JOURNAL OF DAIRY SCIENCE

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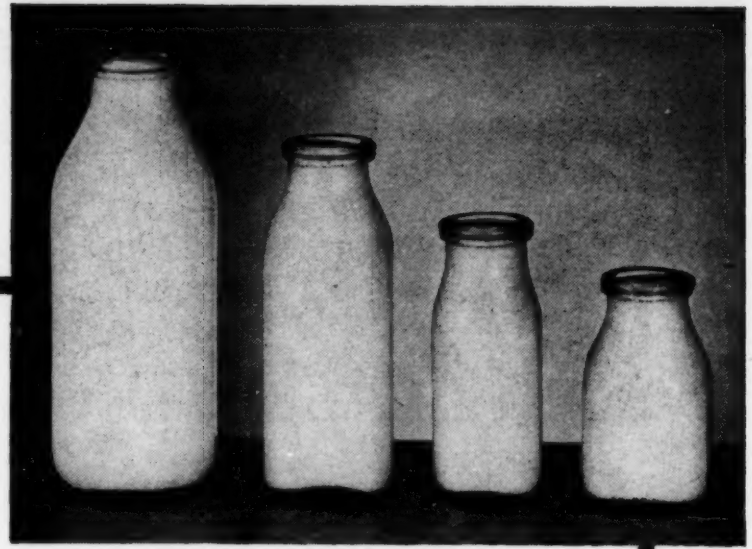

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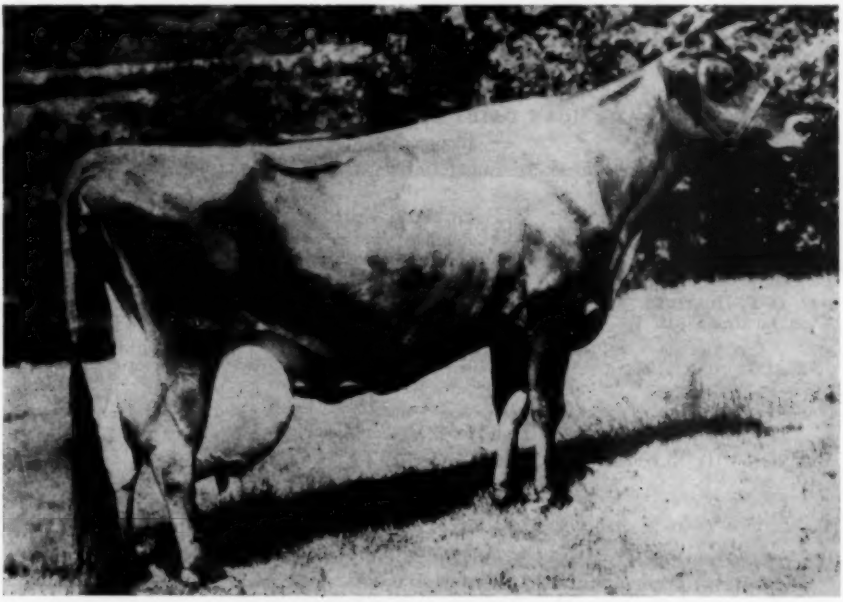

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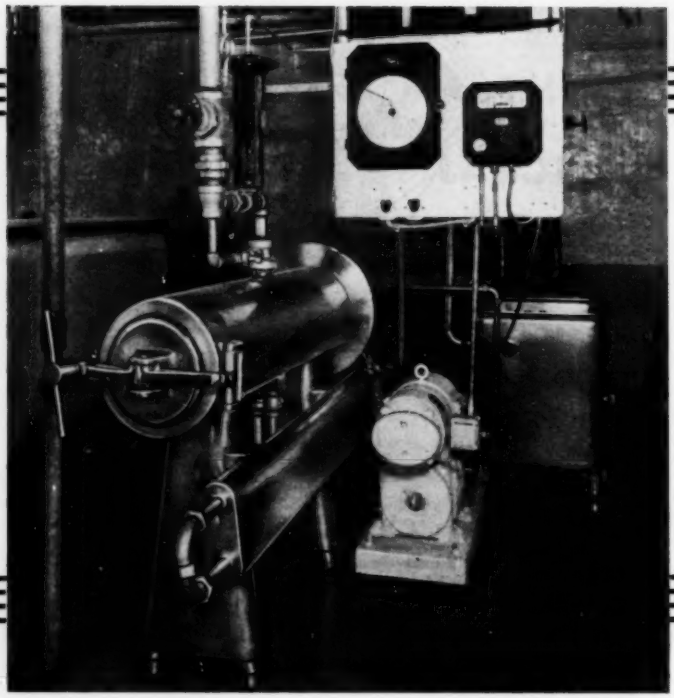

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JOURNAL OF DAIRY SCIENCE

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ALFALFA WITH AND WITHOUT CONCENTRATES FOR MILK PRODUCTION*

VEARL R. SMITH, I. R. JONES, AND J. R. HAAG

Departments of Dairy Husbandry and Agricultural Chemistry
Oregon State College

Numerous investigators (1, 2, 5, 6, 10, 11, 15, 24, 25) have shown that milk and butterfat production on alfalfa hay alone varies from 50 to 85 per cent of that on alfalfa hay and grain rations, with the actual production depending upon the level of grain feeding and the producing ability of the cows. A high producing cow is incapable of consuming sufficient alfalfa hay to meet her energy requirements during the early part of the lactation period; but even in later lactation, when she is consuming adequate nutrients to meet her requirements as judged by the Morrison (22) feeding standard, a cow on alfalfa hay alone does not produce as well as expected from her "total digestible nutrient" intake. The lack of persistency in production suggests a deficiency of some nutrient or nutrients.

Alfalfa, in common with other roughages, is comparatively low in phosphorus. Haag and co-workers (8, 9), conducted metabolism trials with milk cows restricted to alfalfa hay alone and alfalfa hay supplemented with disodium phosphate or bone meal. The cows were always in negative phosphorus balance when alfalfa hay was the only feed but stored phosphorus when phosphorus supplements were given. Huffman and co-workers (14) state that cows on low phosphorus alfalfa hay receive only enough phosphorus for low milk production. Tuff (26) and Eckles and associates (4) have shown that phosphorus-deficient rations reduce milk flow. Growth and lactation studies with rats by Haag (7) and Wright and Haag (27) demonstrated that l-cystine had a supplementary effect when alfalfa leaf meal was fed at levels to provide 9 per cent crude protein. The practical significance of the latter findings, in terms of the feeding value of alfalfa hay produced on sulfur-deficient soils, cannot now be accurately assessed.

Huffman and Duncan (12) fed l-cystine to cows receiving alfalfa hay or alfalfa hay plus starch. While 20 grams of l-cystine per day was reported to have a slight effect in checking the rapid decline in milk production, 40

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grams of l-cystine fed to one cow was accompanied by a sharp drop in milk production and decreased consumption of alfalfa hay. The replacement of 15 pounds of alfalfa hay with an isocaloric (in terms of total digestible nutrients) amount of corn produced a significant increase in milk and fat production. In a recent study these authors (13) report additional results which show that the addition of starch or glucose to alfalfa hay rations failed to increase production. However, the replacement of alfalfa hay with corn or wheat markedly increased production, although the "total digestible nutrient" intake was not increased. On this basis the authors postulate the existence in corn and wheat of an unknown milk-stimulating factor.

That a certain level of fat in the ration appears essential for maximum milk and fat production is indicated by the work of Maynard and co-workers (18, 19, 20, 21). Inasmuch as analysis of the total ether extract of alfalfa hay by Rather (23) showed only 32 per cent true fat, it would seem that cows fed entirely on alfalfa hay might show the effect of the low fat intake on milk and butterfat production.

The study reported in this paper was undertaken to determine the effect on milk production of various concentrates when replacing equal amounts of total digestible nutrients in alfalfa hay rations supplemented with salt and phosphorus.

EXPERIMENTAL PROCEDURE

Five purebred Holstein cows, numbers 322, 334, 336, 341, and 356, and one purebred Ayrshire cow, number 700, were used in this study. The animals were kept in stanchions and turned out in a paved corral about two hours daily. The mangers were constructed to facilitate the accurate measurement of feed intakes. The cows were weighed at the same time each day or week, as discussed later. Water was supplied by individual drinking fountains. The cows had free access to bone meal, to disodium phosphate, and to salt. The daily allowance of good quality second cutting alfalfa hay was weighed into canvas bags and fed in approximately equal portions morning and evening. Hay refused was weighed back daily. Concentrates were also fed in equal portions twice daily. Feed intakes were recorded daily.

Concentrates were substituted for part of the alfalfa hay at 3 to 9 weeks after calving of the individual cows, depending on the decline in milk production and the occurrence of a feed change. The hay allowance was kept a few pounds under what the cows would usually consume so that refused feed would be kept at a minimum. With a few exceptions, feed changes were made simultaneously every 28 days on all cows. The general plan was to follow the period of feeding alfalfa hay alone with two periods of 28 days each when different concentrates replaced 5 or 10 pounds of alfalfa hay, followed in turn by a period in which alfalfa hay alone was fed, and so on throughout the lactation. The total digestible nutrient intakes were main-

tained practically constant during the periods on alfalfa hay alone and when supplemented with concentrates. The digestible protein intakes were kept at the same level as when alfalfa hay alone was fed, by mixing the concentrates with starch or sugar, except when this was impossible with the feeding of concentrates lower in protein than alfalfa.

The cows were milked twice daily by machine. The milk was weighed and aliquot samples were taken at each milking. The milk samples were preserved with corrosive sublimate tablets and butterfat tests were made weekly by the Babcock method.

The chemical analyses as determined for most of the feeds used in this investigation are shown in table 1.

TABLE 1

Analyses of feeds and calculated digestible nutrients

Feed	Moisture	Crude protein	Ether extract	Crude fiber	Ash	Digestible protein	Total digestible nutrients
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Alfalfa hay, first year	6.7	14.7	2.4	30.4	8.0	10.6	52.2
Alfalfa hay, second year	7.0	14.6	2.5	28.8	7.8	10.5	52.6
Blood meal	13.8	80.0	0.5	.....	6.7	68.8	69.9
Beet molasses, dehydrated	2.6	5.0	.....	.....	12.8	2.6	75.0
Beet pulp, dried molasses	6.3	7.2	0.8	21.1	2.6	4.5	74.1
Fish meal, pilchard	9.0	65.9	3.4	.....	15.9	53.4	66.7
Meat meal	7.9	55.0	9.7	.....	22.1	47.3	73.9
Oat groats*	8.5	16.3	5.9	2.8	2.4	14.7	92.5
Peanut meal	9.4	43.8	5.8	14.8	6.4	38.9	73.5
Skim milk powder	3.6	36.6	0.2	.....	7.6	34.8	86.2
Starch, corn	3.0	.....	.....	.....	.....	.....	97.0
Sugar	0.0	.....	.....	.....	.....	.....	100.0
Soybeans	9.4	37.6	20.0	6.5	7.9	33.5	87.9
Soybean meal	11.2	43.0	5.5	7.0	9.5	36.6	75.3
Wheat bran*	9.2	16.1	4.9	9.6	6.0	13.2	69.5
Wheat middlings*	10.0	17.4	5.5	6.8	4.2	14.4	78.4

* Morrison's analyses (22).

Samples of alfalfa hay were taken at regular intervals for analyses. A composite of the samples of a given year's hay crop was subjected to complete chemical analysis. The average digestion coefficients reported by Morrison (22) were used in calculating the digestible protein and total digestible nutrients of the feeds.

RESULTS

Feed consumption. The average daily ration of the 6 cows during the various periods of feeding is shown on figures 1 to 6 inclusive. It will be noted that alfalfa hay alone was fed for variable periods of 3 to 9 weeks at the start of the lactations of the individual cows. The periods thereafter were usually of 4-week duration although there was one 3-week, and an occa-

sional 5-week, period as indicated in the figures. The amount of total digestible nutrients consumed was quite constant througthout the entire lactation and was greater than the requirements except in the early lactation, as shown in the figures. With the exception of periods when dried beet pulp was fed, the calculated total digestible nutrient intake was slightly higher during periods of feeding alfalfa hay only. The digestible protein consumed was from 23 to 60 per cent more than the requirements of the individual cows in early lactation when alfalfa hay only was fed. Inasmuch as production declined as lactation advanced the protein supplied was always greater than the requirement. However, during the periods when sugar, dehydrated molasses, and dried molasses beet pulp were fed, there was a 10 to 20 per cent reduction in the protein intake.

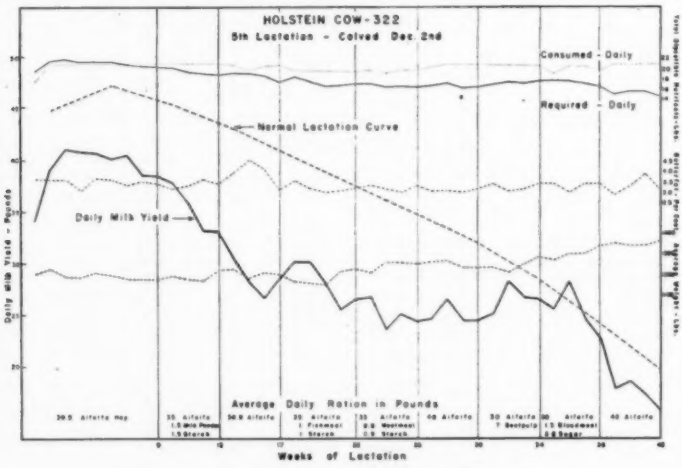

FIG. 1. Feed and production data for Cow 322.

Milk production. The average daily milk production of the individual cows during the experimental lactations is shown by weeks in figures 1 to 6 inclusive. A lactation curve representing the monthly production of 99 purebred Holsteins in the Oregon State College herd (16) is plotted for comparison. Cows that are fed alfalfa hay alone usually have their highest production the first month of lactation, in contrast to the highest production in the second month with normally fed animals. In this experiment, as indicated in the figures, all cows reached their peak production during the first few weeks after calving when alfalfa hay alone was being fed. All cows with the exception of number 700, in addition to the period right after calving, had 3 periods of 28 days each when alfalfa hay alone was fed. A study of the milk yield during these periods shows that it declined more rapidly than when concentrates replaced part of the alfalfa hay.

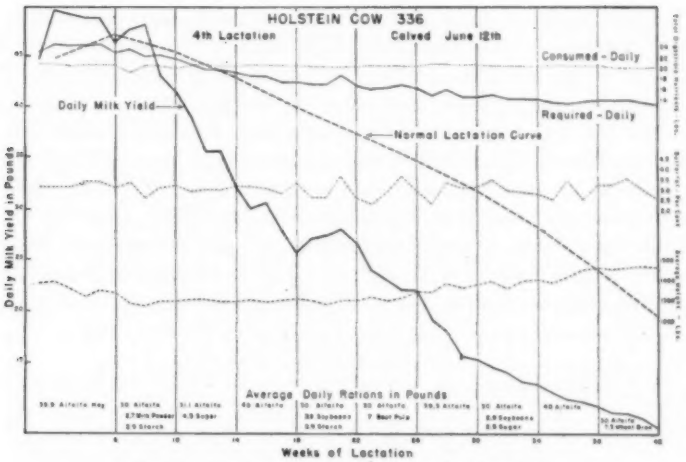

FIG. 2. Feed and production data for Cow 334.

Ground soybeans mixed with starch or sugar were fed during 6 experimental periods of 28 days each to 3 different cows in various stages of lactation, as shown in figures 2, 3 and 5. The replacement rate was 2.9 pounds of ground soybeans and 2.5 pounds corn starch or sugar for 10 pounds of alfalfa hay, a small decrease in calculated total digestible nutrient intake. In 5 of the 6 periods of soybean feeding there was a definite stimulating effect on milk production. Cow number 336 did not respond to soybean feeding after having reached a low level of production at the 30th week of lactation.

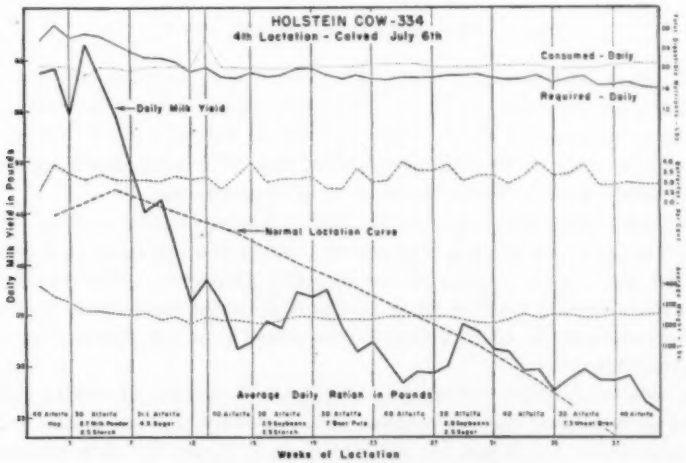

FIG. 3. Feed and production data for Cow 336.

The substitution of skim milk powder and corn starch for alfalfa hay seemed to have an initial stimulating effect on milk flow, figures 1 to 4, especially when 10 pounds of alfalfa hay were replaced in early lactation. Likewise, when the blood meal and sugar mixture was fed, figures 1, 4 and 5, milk production was maintained at a level higher than when alfalfa hay only was consumed. When the total digestible nutrients of 5 pounds of alfalfa hay were replaced by meat meal and starch, figures 1 and 6, and by fish meal and starch, figures 1 and 4, milk production was favorably affected.

When 4.5 pounds of sugar replaced a like amount of total digestible nutrients in skim milk powder and corn starch in early lactation during two 28-day periods, figures 2 and 3, milk production declined about as

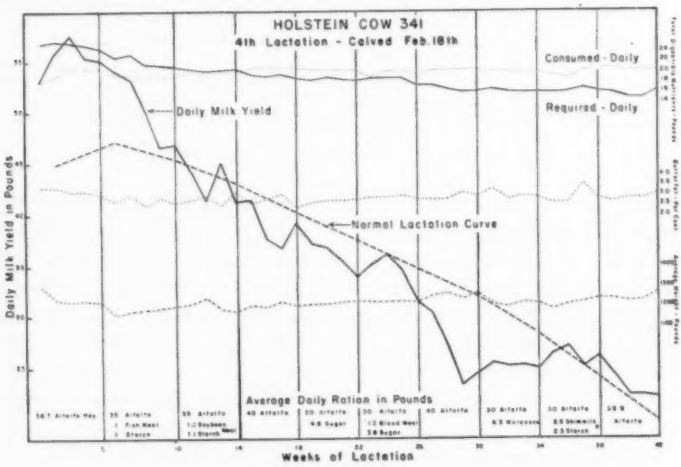

FIG. 4. Feed and production data for Cow 341.

rapidly as on alfalfa only. When 4.8 pounds of sugar replaced 10 pounds of alfalfa hay with one cow in mid-lactation, figure 4, there was no indication of a stimulating effect. The feeding of 6.3 pounds of dehydrated molasses in place of 10 pounds of alfalfa hay to 2 cows, figures 4 and 5, caused only a slightly favorable effect on milk production.

Dried molasses beet pulp was fed during 5 experimental periods. When 7 or 7.3 pounds of beet pulp replaced 10 pounds of alfalfa hay in 3 periods, figures 1 and 5, milk production was definitely improved. However, during two periods, figures 2 and 3, when beet pulp followed the feeding of ground soybeans and starch, milk production decreased at a rate faster than when soybeans were fed.

In this study other concentrates replacing 5 pounds of alfalfa hay included soybean meal for two periods, figures 4 and 6, and oat groats, peanut meal and wheat middlings for one period each, figure 6. Milk production

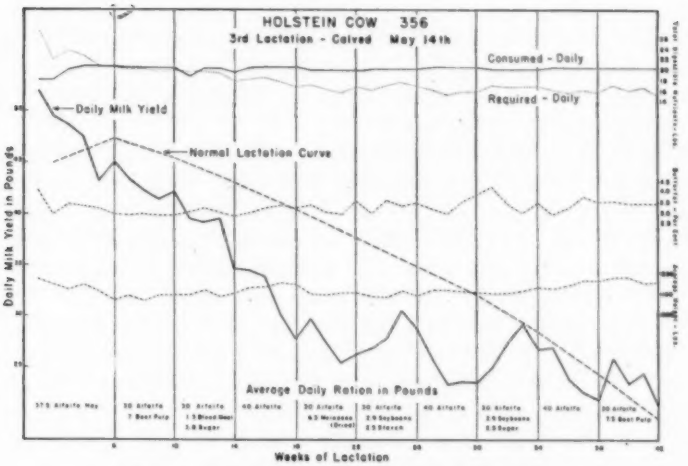

FIG. 5. Feed and production data for Cow 356.

in all cases was maintained at a higher value than expected on alfalfa hay only.

Per cent of butterfat. The butterfat tests of the weekly composite milk samples are presented graphically in figures 1 to 6. While there was considerable weekly variation in the tests, it is doubtful if any effect can be attributed to the different rations. Thus it is found that, during six 28-day experimental periods when ground soybeans were fed, the average butterfat test was 3.26 per cent as compared to an average test of 3.25 per cent for

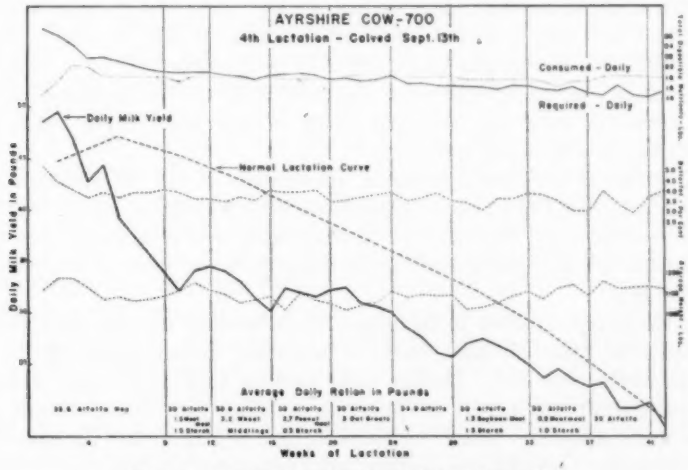

FIG. 6. Feed and production data for Cow 700.

the 12 experimental periods just preceding and following the soybean feeding periods. The level of fat in the ration varied from 2.23 per cent of the dry matter when alfalfa hay and sugar were fed, to 3.98 per cent of the dry matter when soybeans and starch replaced a like amount of digestible nutrients in 10 pounds of alfalfa hay.

Body weights. The weight data for the individual cows are presented graphically in figures 1 to 6 inclusive. The weekly weights plotted are the average of daily weights for cows 334, 336, and 356, figures 2, 3 and 5 respectively, throughout the lactation; for cow 322 after the 27th week and for cow 700 after the 39th week. The fluctuations in body weight are much greater in early lactation of the cows which were weighed weekly.

Cow 334, figure 2, reached a fairly stationary weight level at about the 12th week of lactation and kept at the same level until about the 22nd week, followed by a gradual increase as milk production decreased. She did not show a decrease in weight when ground soybeans stimulated milk production at the 18th week of lactation. Actually a small increase in weight due to greater dry matter consumption and increased body fill should be expected in periods of feeding on alfalfa hay only.

There was no large weight change with cow 356, figure 5, during the periods of ground soybean feeding when milk production was noticeably stimulated. The weekly weight curve for cow 341 was fairly uniform after daily weights were averaged. The weight curves of cows 322 and 700 are too variable to show definite effects and illustrate the point that frequent weighings are necessary to compensate for normal daily variations.

DISCUSSION AND RESULTS

This study shows that good producing dairy cows do not utilize a ration of alfalfa hay supplemented only with minerals as well as when part of the alfalfa hay is replaced by calculated equal amounts of total digestible nutrients in various concentrates. As graphically shown in figures 1 to 6, the decline in milk flow was practically always more rapid when only alfalfa hay and minerals were fed.

A study of the month-to-month production of the 99 Holstein cows plotted in figures 1 to 6 as the normal lactation curve shows a fairly uniform rate of decline from the third to the tenth month of lactation, the average production for any particular month being 92.6 per cent of the previous month (16).

In the study reported in this paper there were 17 mid-lactation periods (between the 12th and 42nd weeks of lactation) during which alfalfa hay and minerals were fed. During these periods milk production averaged 84.5 per cent of that during the previous periods, a production considerably lower than expected. It will be noted in figures 1 to 6 that the calculated total digestible nutrient consumption met or exceeded the requirements after the 12th week of lactation.

There were 25 periods of feeding in mid-lactation during which part of the alfalfa hay ration was replaced with a calculated equal amount of total digestible nutrients in various concentrates. Milk production during these periods averaged 96.2 per cent of the previous periods. This represents a higher production than normally expected, and significantly better than when alfalfa hay and minerals only were fed. The highest production was obtained during 6 periods when ground soybeans were fed, the milk flow averaging 101.2 per cent of the previous periods. Whether the good results from feeding ground soybeans was due to the increased fat intake cannot be definitely stated at this time.

As indicated in figures 1 to 6, the amount of alfalfa hay replaced was either 5 or 10 pounds daily. A summary of the 18 mid-lactation periods, during which an average of 5.1 pounds of calculated total digestible nutrients in 7 different concentrates replaced 5.2 pounds in 10 pounds of alfalfa hay, shows that the milk yield averaged 97.0 per cent of the previous periods. During the 7 periods when 2.6 pounds of calculated total digestible nutrients in 5 pounds of alfalfa hay were replaced with an average of 2.1 pounds of total digestible nutrients in 7 different concentrates, milk yield averaged 94.2 per cent of the previous periods.

From the facts that cows do not produce as well as expected from their total digestible nutrient intakes on alfalfa hay supplemented only with salt and phosphorus, and that each of 12 different concentrates fed alone or with starch or sugar gave superior production results when replacing an approximately equal amount of calculated total digestible nutrients in alfalfa hay, it appears that the production value for dairy cows of an alfalfa hay ration is overrated by the total digestible nutrient method of calculating feeding value.

A study of the results in terms of the net energy system of feed evaluation (22) indicates that this more nearly gives a ration of alfalfa and minerals only its proper production value. On this basis, during the 18 periods when 10 pounds of alfalfa hay were replaced by concentrates, there was an average calculated increase of 4.1 per cent in net energy intake, which would account in part for the higher than expected milk flow—97 per cent of the previous periods. During the 7 periods when concentrates replaced the nutrients in 5 pounds of alfalfa, the average net energy intake was increased 3.7 per cent, and the milk flow averaged 94 per cent of the previous periods. The five Holstein cows during mid-lactation periods on alfalfa hay and minerals only consumed 16.6 Therms of net energy daily, which according to recommended standards (22) was sufficient for their body maintenance and the production of an average of 27.8 pounds of 4 per cent milk. The actual average production was 21.8 pounds of calculated 4 per cent milk, a decline of 7 pounds daily from the previous periods when concentrates were fed. It would seem that concentrates fed at a low level

with large quantities of alfalfa hay have a net energy value for milk production as high as that usually assigned to them, even when compared to the relatively low net energy value given to alfalfa hay. The greater efficiency of converting feed energy into milk energy by cows maintained for complete lactations on alfalfa hay and limited concentrates compared to alfalfa hay only has also been shown in an earlier report (17).

The results obtained with various concentrates give no indication of a difference in milk yield due to improvement in quality of protein. The best results were obtained with ground soybeans, followed in turn by high and medium protein feeds from plant sources, dried molasses beet pulp, animal protein feeds, dehydrated molasses and sugar.

In general our results appear to be strikingly similar to those recently reported by Huffman and Duncan (13).

The possible effect of the ration fed on the butterfat test of composite weekly samples is difficult to evaluate. Variations in test were quite marked and are complicated by possible seasonal effects as the cows freshened in different months, as noted on figures 1 to 6.

CONCLUSIONS

The total digestible nutrient system of feed evaluation considerably overrates the production value of good alfalfa hay supplemented only with salt and phosphorus when fed in large amounts to good dairy cows.

The production results on alfalfa hay only are not quite as high as expected when calculated on the net energy basis of 41.5 Therms per 100 pounds.

The replacement of 13 to 25 per cent of the total digestible nutrients in alfalfa hay rations with various concentrates allowed cows to produce at a normal level.

The feeding of ground soybeans with alfalfa hay stimulated milk flow to the greatest extent of any concentrate used.

There is no indication that the better milk production with concentrate feeding is due to improvement in quality of protein. Production was less with molasses or sugar than with the other concentrates fed.

There was no consistent, measurable effect of the concentrates fed on the per cent of butterfat in the milk or on the body weights of the cows.

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RELIABILITY OF AVERAGES OF DIFFERENT NUMBERS OF LACTATION RECORDS FOR COMPARING DAIRY COWS[1]

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This investigation was undertaken to determine a fair basis for comparing the productiveness of dairy cows with varying numbers of records. The problem concerns the values of various combinations of production records, both for predicting a cow's future records and for evaluating her transmitting ability. A previous publication (2) pointed out the importance of using all the records available rather than selected records.

DATA STUDIED

Through the courtesy of H. W. Norton, Jr., Superintendent of Advanced Registry for the Holstein-Friesian Association of America, material from the Herd Improvement Register Yearbooks up to and including Volume 10 was made available early in 1939. The data used included:

1. All butterfat records of all (454) cows[3] which had completed six or more lactations.

2. The butterfat record of all (979) those dams and daughters (of the six-record cows) which had completed at least one record of production.

3. The first two butterfat records of all (661) dams and daughters (of the six-record cows) which had completed at least two records.

4. Identification of each six-record cow with the herd in which all, or the majority, of her records were made. There were 98 of these herds.

All records were brought to a mature "B" basis (milked thrice daily) by use of the conversion factors listed in the Holstein Red Books. Only the production during the first 365 days was used. Complete lactations which were less than 365 days in length were used as they were, without adjustment for length.

The primary statistical ingredients of the analyses were gross and intra-herd standard deviations and correlations between different records of the same cow, and between parent and offspring. Correlations between various combinations of records were calculated by formulae involving path coefficients (15).

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[2] This study was made while the author was Research Fellow at Iowa State College. He is indebted to Dr. J. L. Lush and to Dr. C. Y. Cannon for guidance during the conduct of this study and for valuable criticism of the manuscript.

[3] These cows will be referred to as "six-record" cows; 181 of them had a seventh record.

FINDINGS

Means and Standard Deviations

Table 1 shows the means and standard deviations.

When butterfat records collected from many herds are analyzed as a single population, the standard deviations and the correlations will be materially larger than those for the same records analyzed on an intra-herd basis if the herd averages differ from each other more than if all herds were random samples from the same population. The formula $1 - \frac{\text{intra-herd } \sigma^2}{\text{inter-herd } \sigma^2}$, when applied to the present data, shows that 41 and 51 per cent, respectively, of the gross variance among single records in the six-record and seven-record studies was caused by whatever made the real differences between the herd averages.

TABLE 1

Means and standard deviations (in pounds) for butterfat records of 454 6-record and 181 7-record cows

Records	6-record cows			7-record cows		
	Mean	Std. deviation		Mean	Std. deviation	
		Gross	Intra-herd		Gross	Intra-herd
First	500	114	93	491	114	88
Second	493	116	87	478	109	80
Third	496	113	86	483	107	74
Fourth	491	117	86	485	114	71
Fifth	481	114	91	482	112	80
Sixth	481	126	96	486	125	86
Seventh	.....	.....	.....	465	115	82

Repeatability of Records of the Same Cow

Table 2 shows the correlations between different records of the same cow, the correlations being grouped and averaged according to proximity of records. The average values (gross $r = 0.41 \pm 0.04$, intra-herd $r = 0.29 \pm 0.04$) found in the six-record analysis are near the lowest levels which have been reported from other studies. Those of the seven-record study (gross $r = 0.38 \pm 0.06$, intra-herd $r = 0.23 \pm 0.07$) are still smaller. While two analyses (3, 6) of somewhat similarly selected data have shown correlations only a little larger than these, values more of the order of 0.4 to 0.7, where herd differences were left in the data, and 0.3 to 0.5, on an intra-herd basis, have generally been found.

The lower repeatability found here may be due in part to the restriction of the study to cows which had at least six records. Doubtless some selection against low production took place in these herds, especially after the first and second records. The discarding of cows with extremely low first records would reduce the standard deviations of those early records and would cause

TABLE 2

Intra-herd correlations for butterfat records of 454 6-record cows and 181 7-record cows

Records compared*		Correlation coefficients (intra-herd)	
		6-record cows	7-record cows
Adjacent	A & B	0.27	0.10
	B & C	0.28	0.13
	C & D	0.38	0.36
	D & E	0.37	0.44
	E & F	0.44	0.40
	F & G	.....	0.34
	Average	0.35	0.29
One record intervening	A & C	0.29	0.26
	B & D	0.25	0.12
	C & E	0.30	0.29
	D & F	0.37	0.34
	E & G	.....	0.23
	Average	0.30	0.25
Two records intervening	A & D	0.24	0.14
	B & E	0.22	0.24
	C & F	0.27	0.24
	D & G	.....	0.25
	Average	0.24	0.22
Three records intervening	A & E	0.28	0.22
	B & F	0.15	0.13
	C & G	.....	0.15
	Average	0.22	0.17
Four records intervening	A & F	0.25	0.19
	B & G	.....	0.16
	Average	0.25	0.17
Five records intervening	A & G	.....	0.13
Average of all correlations		0.29	0.23

* Records are designated by letters as follows: A = first record; B = second; C = third; D = fourth; E = fifth; F = sixth; G = seventh.

the mean of the first records to be a little higher than the mean of the later records. Table 1 shows a change in the means, as expected on this hypothesis, but does not show a reduction in the standard deviation of first records.

The chi-square method outlined by Paterson (11) showed that the correlations in the six-record study differed among themselves more ($P < 0.01$) than if all had been samples from the same population. Scrutiny of them suggests that there is a general tendency for records made close together in time to be more highly correlated than records separated by long time intervals, and that there is also some tendency for later records to be more closely correlated with each other than the earlier records are with each other.

Prediction of Future Production

(a) *Prediction of single records*

The correlations between a single record and various combinations of preceding records made by the same cow are shown in table 3.

The formula[4]

$$r_{X_nY} = r\sqrt{\frac{n}{1+(n-1)r}}$$

gave the series of expected values shown in the last column of table 3. These figures in the last column describe with considerable accuracy how these correlations actually do increase as n increases, but at an ever decreasing rate. The major gain is made when a second record becomes available to use along with the first. The addition of a third record is of some further value, but records beyond the third add only a little to the prediction value of the average. Column II of table 3 is for consecutive records only and not necessarily starting with the first. It contains, therefore, more of the effects of

TABLE 3

Correlations of single and average records with subsequent single records for 454 6-record cows

Records correlated with subsequent single record	Correlations			
	Observed*			Expected r_{X_nY}
	I	II	III	
Single records	0.27	0.35	0.29	0.29
Averages of two records	0.36	0.41	0.37	0.36
Averages of three records	0.40	0.43	0.41	0.40
Averages of four records	0.43	0.44	0.44	0.42
Averages of five records	0.46	0.46	0.46	0.44

* The observed values in Column I are for consecutive records only and start always with the first record. (The first value, 0.27, is for r_{AB}; the second, 0.36, is for $r_{\overline{AB}\cdot C}$; etc.) In column II the values are also for consecutive records only but are not limited to starting always with the first record. (The first value, 0.35, is the average of r_{AB}, r_{BC}, r_{CD}, . . ., etc.; the second, 0.41, is the average of $r_{\overline{AB}\cdot C}$, $r_{\overline{BC}\cdot D}$, $r_{\overline{CD}\cdot E}$, . . ., etc.; etc.). In column III the values are averages of all the possible correlations for both consecutive and nonconsecutive records.

age and proximity than do the other columns. Because of this, it would be expected to show somewhat higher values, and it actually does.

(b) *Prediction of average future production*

Observed and expected correlations between the first three records, singly and in the various possible combinations, and the average of the last three records were calculated. They ranged from 0.25 to 0.55 and showed the general trend to an increase in prediction value as more records are included in the basis for prediction.

[4] In this formula, X_n is the average of n preceding records, Y is the succeeding record and r is the average intra-herd repeatability of records of the same cow (0.29 in these data). The observed correlations would reduce to this if all the r's and standard deviations were identical.

Estimation of Real Producing Ability

The approach to this problem is illustrated in figure 1. A cow's real ability is represented by W. W is permanent and unvarying and includes, besides a cow's genuinely inherent characteristics, also such environmental effects as are permanent throughout her lifetime (*i.e.*, environmental circumstances which were not the same for her as for her herd mates, and which affected the size of all her records equally).

Y_∞ is what a cow's production records would average if it were possible for her to complete an infinite number of records. It is identical with W.

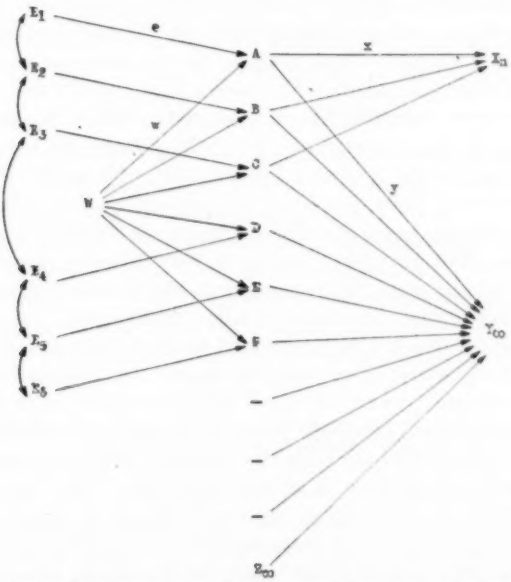

FIG. 1. Relationship between a cow's records and her real producing ability. A, B, . . ., F are actual records; X_n is an average of n of those. Y_∞ is the average of an infinite number of records. W is the cow's real ability and E_1, E_2, . . ., E_6 are environmental influences which vary from lactation to lactation.

Y_∞, of course, cannot be observed directly but can be predicted from X_n, which approaches Y_∞ as n becomes large. The correlation between X_n and Y_∞ depends on the correlations between the records and on the standard deviations of the records. The correlations between the records in turn depend largely on having W as a common cause of them all, but also in part on correlations between some of the environments. The effect of proximity, for example, results from the correlation between E_1 and E_2 or between E_2 and E_3, being larger than that between E_1 and E_3 or between either of those and E_6.

The prediction equation for estimating real ability (W or Y_∞) is:

$$\overline{Y_\infty} = \text{herd average} + r_{X_n Y_\infty} \frac{\sigma_{Y_\infty}}{\sigma_{X_n}} \text{ times (cow's ave.} - \text{herd ave.)}$$

The path coefficient method was used to calculate expected values for the regression coefficient, $r_{X_n Y_\infty} \frac{\sigma_{Y_\infty}}{\sigma_{X_n}}$, for the various averages of n records. This turns out to be

$$\frac{n(r - e^2 r_{EE})}{1 + (n-1)r} \text{ or } \frac{nw^2}{1 + (n-1)r}.$$

When $e^2 r_{EE}$ equals zero, this is the same formula as that derived by Berge (1), Lauprecht and Münzner (7), and Lush *et al.* (9), and recommended for use in estimating a cow's real ability by Lush (8) and by Berry and Lush (2). Necessarily r must be the average of the correlations actually observed among the n records included in X_n (unless an r known from other data or deduced from other considerations is to be used). The $e^2 r_{EE}$ term takes care of such circumstances as proximity, which may make the average r larger than w^2, especially when n is only two or three. Naturally the $e^2 r_{EE}$ term would be very small when n is large, if it depends solely on circumstances which prevail during parts of adjacent lactations but do not persist into a third lactation. For example, when $n = 3$, two-thirds of the correlations in the average r are between adjacent records but when $n = 6$, only one-third of the fifteen correlations are between adjacent records.

The magnitude of $e^2 r_{EE}$ was estimated to be of the order of 0.03 to 0.09 by comparing the correlations between records on the basis of proximity (table 2). A similar proximity effect was observed in correlations calculated by Gowen (5) and by Seath (13). It is concluded, therefore, that w^2 has a value of 0.03 to 0.09 less than the average intra-herd correlation (0.29 in these data) between records of the same cow. The accuracy of this approximation is subject to sampling errors among the correlations and to inability to be sure that even those records separated by as many as five intervening lactations were made under environments as uncorrelated as would be the case with lactations separated by ten, twenty, or more intervening lactations, if such could be observed. This becomes a matter of defining "permanent" when stating that W includes the permanent effects of environment peculiar to a particular cow in all her lactations.

Expected values for the regression coefficient at four levels of $e^2 r_{EE}$ were calculated for all six values of n for the six-record cows, using 0.29 as r in all cases. These coefficients are shown in table 4 and are the fractions of the apparent superiority of a cow's average of n records over the herd average, which need to be added to the herd average to constitute an estimate of her real ability.

Since $r = w^2 + e^2 r_{EE}$, differences between the four series of values (table 4) arise solely from differences in the fraction of r which is supposed to be due to w^2. Because this does not affect the denominator, the *relative* gain with

TABLE 4

Expected values for the regression coefficient for predicting real producing ability from averages of n consecutive records when r = 0.29

No. of records (n) in the ave.	Regression coefficients			
	$e^2r_{EE}=0$	$e^2r_{EE}=0.03$	$e^2r_{EE}=0.06$	$e^2r_{EE}=0.09$
1	0.29	0.26	0.23	0.20
2	0.45	0.40	0.36	0.31
3	0.55	0.49	0.44	0.38
4	0.62	0.56	0.49	0.43
5	0.67	0.60	0.53	0.46
6	0.71	0.64	0.56	0.49

increases in n is the same for all four conditions, but the absolute size of the increase is larger the more nearly r results from w^2 alone.

Estimation of Breeding Worth

The 454 six-record cows had 979 daughters (or dams[5]) with at least one record. Of these, 661 had a second record. Gross and intra-herd correlations between these records are shown in table 5.

The intra-herd correlations between the single records of cows and their daughters or dams are prevailingly positive but small. The six daughter-dam correlations in the one-record daughter or dam analysis range from 0.03 to 0.13, and average 0.07, a value which is nearly six times its sampling error. This value is in excellent agreement with the finding of Seath (13) of an

TABLE 5

Gross and intra-herd correlations (r) for the records of 6-record cows and their 1-record and 2-record daughters or dams

Records* correlated	Cows and 1-record daughters or dams		Cows and 2-record daughters or dams	
	Gross r	Intra-herd r	Gross r	Intra-herd r
L & A	0.202	0.091	0.204	0.062
L & B	0.171	0.033	0.194	0.010
L & C	0.231	0.128	0.240	0.067
L & D	0.199	0.060	0.234	0.035
L & E	0.160	0.030	0.180	0.009
L & F	0.154	0.060	0.156	0.016
Average	0.186	0.067	0.201	0.031
M & A	.....	.....	0.160	0.093
M & B	.....	.....	0.190	0.038
M & C	.....	.....	0.192	0.070
M & D	.....	.....	0.231	0.126
M & E	.....	.....	0.157	−0.004
M & F	.....	.....	0.194	0.022
Average	.....	.....	0.187	0.058
L & M	.....	.....	0.524	0.471

* Cow records: A = first; B = second; C = third; D = fourth; E = fifth; F = sixth. Daughter or dam records: L = first; M = second.

[5] Dams were included because the relationship is a parent-offspring one in either case.

intra-sire correlation of 0.07 derived from an analysis of the first available butterfat record on each animal in 620 daughter-dam pairs (Iowa C.T.A. data). The six intra-herd correlations between a cow's records and her daughter's or dam's second record range from – 0.004 to + 0.126 and average 0.06. There seems to be no regular trend toward the earlier or the later records of a cow being better than other records as a guide to her daughter's production.

The difference between gross and intra-herd correlations is due to the herd effect. The importance of this in dairy data is sometimes overlooked. Its magnitude here in the one-record daughter analysis is indicated by the difference between the average value 0.19, where herd effects have been left in the data, and 0.07, where they have been eliminated.

TABLE 6

Observed and expected correlations for averages of a dam's records with her daughter's records

Average of dam's record	Correlation coefficients			
	979 1-record daughters		Average of first and second records of 661 2-record daughters	
	Observed	Expected*	Observed	Expected*
A	0.091	0.067	0.090	0.052
A & B	0.079	0.088	0.076	0.065
A, B & C	0.113	0.097	0.089	0.072
A, B, C & D	0.114	0.103	0.106	0.076
A, B, C, D & E	0.105	0.108	0.090	0.079
A, B, C, D, E & F	0.106	0.111	0.082	0.082

* On the assumption that the daughter's records were correlated with each of the dam's records, 0.067 and 0.052 in the 1-record and 2-record studies respectively. These two values are the actual averages of the correlations observed in the two studies and are hence the expected values when only one record of a dam is used.

The correlations between averages of a dam's records and of the first two records of her daughter are shown in table 6. The expected values increase at a continuously decreasing rate as more records are used for prediction. These expected increases are small, however, and the observed values do not show them with certainty. The observed values, of course, are attended by considerable sampling error.

Relationship between Real Producing Ability and Breeding Worth

The biometric relationships involved between a cow's real producing ability and her transmitting ability are illustrated in figure 2. Here p is the correlation between W and W_1; that is, it is the correlation between a daughter's real producing ability and that of her dam. If mating were entirely at random and if milk production were completely hereditary in the narrowest sense of the word (all variations additively genetic), then p would equal one-half (Wright (14)).

From the equation $q = wpw_1$ (which assumes that $r_{E_1E_2} = \text{zero}^6$), p is calculated to have a value ranging from 0.27 to 0.35. (This uses for ww_1 values from 0.26 to 0.20, as appear in the upper line in the last three columns of table 4.) Compared to the 0.5 for perfect heritability (and random mating), this approximation indicates that about 54 to 70 per cent of those characteristics conditioning a cow's real ability (W) would be expected to

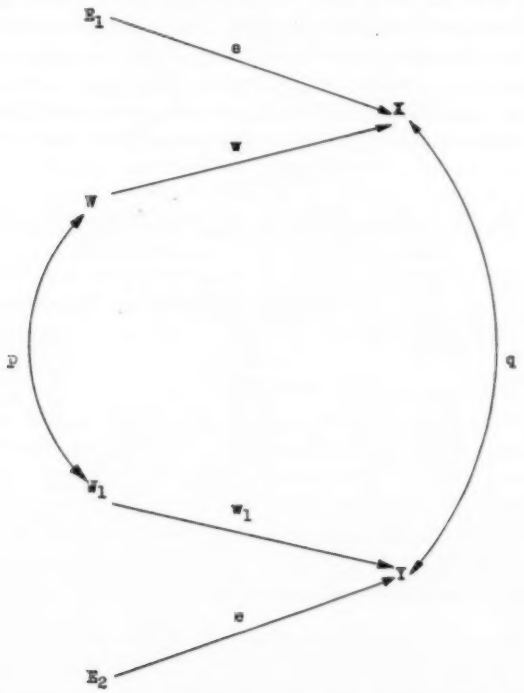

FIG. 2. Biometric relations between parent and offspring. X is a dam's record; Y is her daughter's record. E_1 and E_2 are environmental influences which vary from lactation to lactation. W and W_1 represent real producing ability. Correlations between W and W_1 and between X and Y are indicated by p and q respectively.

be transmitted. Only one-half of this, however, would be expected to be evidenced in the daughter because one-half of the latter's inheritance comes from her sire.

INTERPRETATION

The confidence with which the findings of this investigation may be ex-

[6] This seems a reasonably safe assumption when the data are analyzed on an intra-herd basis but usually would not be true if the data from several herds were analyzed as if they were a single homogeneous population.

tended to all dairy cows involves primarily the question of whether the degree of repeatability found in these data is typical. Most investigators of the tendency for records of the same cow to repeat themselves have reported intra-herd correlations of the order of 0.3 to 0.5. The lower values found here are believed to result (at least in part) from the selection of the cows, a view supported by the correlation values found in investigations (3, 6) of similarly selected data. To be included in these analyses, cows must have completed at least six records. In so far as voluntary selection caused the less productive cows to be absent from the data, it would tend to exclude the lower portion of the frequency distribution, especially in the early lactations, and this would decrease the observed correlation. It would appear that the so-called "age effect" on repeatability observed here was at

TABLE 7

Estimating the real producing ability and the transmitting ability of a cow whose M.E. average of n records is 100 pounds above the herd average when $r = 0.4$, $w^2 = 0.31$ to 0.37 and $q = 0.10$

No. of records, n	Apparent superiority of average	Probable superiority in real ability = $\frac{nw^2}{1+(n-1)r}$	Proportion, $\frac{nq}{1+(n-1)r}$, of apparent superiority expected to be transmitted*
1	100	31–37	10
2	100	44–53	14
3	100	52–62	16
4	100	56–67	18
5	100	60–71	19
6	100	62–74	20

* This to appear in the daughters if only the dams are selected, in which case the breeding value of the sires would be expected to average the same as that of the generation in which they were born. Twice this to show up if the sire was so selected that his transmitting ability was equal to that of the dam.

least partly due to the selection of the data—a view which is supported by the absence of an age effect in 1,741 records analyzed by Gowen (5).

There appears no reason to believe, however, that the "proximity effect" would disappear by the inclusion of all cows. It has already been pointed out that the unselected data of other investigators have shown higher correlations between adjacent records than between those separated by one or more lactations and that the differences observed are similar to the ones reported here. Also it is reasonable to suppose that some special conditions of health or environment would in many cases affect an animal during a period of time extending over parts of consecutive records, but not all through the animal's life—perhaps not often over periods long enough to include all of one lactation and parts of both the preceding and the following ones.

It is difficult to estimate what effect the inclusion of all cows from the 98 herds and all daughters of those cows would have on the intra-herd corre-

lation of 0.07 observed between records of dam and daughter. Assuming that closely proportional increases in q and r would result from the inclusion of all cows (regardless of number of completed records), it is suggested that values of 0.10 and 0.40, respectively, might be typical. Doubling the former value would give a figure for heritability of 0.20. This is just slightly lower than that found by Lush and coworkers (10) in an analysis of D.H.I.A. and H.I.R. data.

It is assumed, therefore (by reasoning not rigorously supported in all respects by adequate data), that the following approximations: $r = 0.4$, $w^2 = 0.31$ to 0.37, $q = 0.10$, are more nearly indicative of the relationships actually existing between records of all cows in a herd than the values $r = 0.29$, $w^2 = 0.20$ to 0.26, $q = 0.07$ deduced from the special data of this investigation. Since, however, both sets of values depict substantially the same rate of increase in the reliability of averages of n records, it would appear to make little difference which is used as long as cows are compared by the same standard of values.

The application of these ideas of real producing ability and transmitting ability to estimating the ability of a cow whose average of n mature equivalent records is 100 pounds above the herd average is shown in table 7.

SUMMARY

Formulae which describe the relative reliability for prediction purposes of averages based on different numbers of records have been presented.

The major increase in reliability occurs when a second record becomes available to use along with the first. The addition of a third record adds considerably to the reliability of the estimate. Records beyond the third do contribute more information but so little that they scarcely appear to be worth waiting for before estimating the consequences of keeping or culling the cow herself or her offspring.

It is concluded that cows can be compared fairly, without bias because of differences in number of records, by use of the following prediction equations:

(1) Real producing ability (W) = herd average + $\frac{nw^2}{1+(n-1)r}$ times (cow's average – herd average).

(2) Transmitting ability or breeding value = herd average + $\frac{2nq}{1+(n-1)r}$ times (cow's average – herd average).

In these equations n is the number of records in the cow's average, r is the average intra-herd repeatability of records of the same cow (usually of the order of 0.3 to 0.5), q is the average intra-herd correlation between dam and daughter records (probably not far from 0.1 generally), and w^2, which is that part of r left after the effects of proximity are removed, is believed to have a value of approximately 0.03 to 0.09 less than r.

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LACTIC ACID IN DAIRY PRODUCTS. III. THE EFFECT OF HEAT ON TOTAL ACID AND LACTIC ACID PRODUCTION AND ON LACTOSE DESTRUCTION[1]

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Proper application of lactic acid measurements to the grading of concentrated milk requires thorough knowledge as to the possible sources of lactic acid in these products and the relative amount of the acid which is derived from the various sources. In general, the lactic acid of concentrated milk products, as measured either by the Hillig colorimetric procedure (4, 6, 7) or the aldehyde method of Troy and Sharp (14), may be said to result from four sources: (a) the blank value for strictly fresh raw milk equivalent to 1 to 3 mg. of lactic acid per 100 gm. milk; (b) the lactic acid resulting from microbiological changes in the raw milk; (c) the lactic acid resulting from processing procedures involving high temperatures; (d) the lactic acid resulting from storage of the manufactured product. The first two sources listed were considered in the preceding papers of this series (4, 5), and this paper is to deal with the third source, *i.e.*, the lactic acid resulting from high-temperature heat treatment. The influence of storage will be considered in a subsequent paper.

Acid production in milk as a result of heat treatment has been observed by many workers (2, 3, 8, 9, 10, 11, 15, 16, 17, 18), and it is now generally agreed that the amount of acid formed is largely dependent on the temperature and time of heating. However, the two questions which have concerned various workers interested in this subject are (a) the source of the acid, and (b) the specific acid or acids involved.

On the basis of available information, it may be assumed with some certainty that the major source of the acid formed when milk is heated is lactose. The destruction of a portion of the lactose when milk is heated has been observed by Leeds (11), Whittier and Benton (17), Kometiani (10), Kass and Palmer (8, 9) and others. Whittier and Benton (17) report an analysis on a sample of milk indicating that, on a mol basis, the lactose loss (resulting from heat) was sufficient to account for more than four times the amount of acid formed. Furthermore, they observed that the addition of five per cent of lactose to milk resulted in practically twice as much heat-produced acid as in the normal milk. Kometiani (10) heated milk for 30 minutes at 100°, 110°, and 120° C. and obtained results for pH which were

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lower than the control by 0.08, 0.27, and 0.46, respectively. For these same samples, the titrable acidity expressed as lactic acid increased 0.009, 0.036, and 0.075 per cent, respectively, and decreases in lactose amounted 28, 92 and 265 mg. per 100 ml.

The specific acids formed when milk is heated have not been definitely established. Leeds (11) in 1891 suggested that the acid produced by heat destruction of lactose is probably glucic acid. Whittier and Benton (17) observed that the common curve obtained from heating milk in sealed cans at 90°, 95°, 110°, and 120° C. indicated that the buffer action exerted by the milk samples was practically the same at all temperatures; and, therefore, they considered it likely that the same acids in very nearly the same proportions were formed in each case. They also observed that the steam distillate from heated milk gave characteristic tests for formic acid.

Kometiani (10) found formic and lactic acids to result from heating milk and that the amount of these acids corresponded to the amount of decomposed lactose. Furthermore, his quantitative measurements revealed that the amount of lactic acid formed was four to five times the amount of formic acid. Milk heated at 120° C. for 30 minutes contained 202.3 mg. lactic acid and 42.1 mg. formic acid per 100 ml. Troy and Sharp (14) show graphically a marked increase in lactic acid when milk was heated for a prolonged period at 100° C., and yet a much greater increase in the same milk when it was partially neutralized. For the normal milk, heating for 6- and 10-hour periods gave iodine titers approximating 28 and 35 mg. lactic acid per 100 gm. respectively.

PROCEDURE

Milk used for these experiments was obtained from the College Creamery and was either the regular commercial homogenized whole milk or fresh raw skim milk. The milk was sealed in "Canco" No. 2 plain cans (American Can Co.) having a capacity of approximately 500 ml. Heating at 100° C. was accomplished by submerging the cans in a boiling water bath, whereas an autoclave was used for these samples heated at 116° C. At definite intervals, the cans were removed and placed immediately in cold running water for cooling. The milk was then held at about 3° C. until examined. During the heating periods, the cans were shaken at 30-minute intervals so as to insure uniform heating of the milk and also to aid in the prevention of heat coagulation.

Whey used in one series of experiments was prepared by rennet-coagulation of the casein of freshly separated skim milk, and removing the coagulum by filtration through several thicknesses of cheese cloth.

pH determinations and electrometric titrations were made using a Beckman Type E electrode and Type G pH meter. The titrations were made on 50-ml. samples with N/10 NaOH and using pH 8.3 as the end point.

Lactic acid was determined essentially by the Hillig colorimetric pro-

cedure (6, 7) with slight modifications (4). Additional modifications were found necessary in preparing the whey for extraction since the standard procedure of precipitation resulted in a serum which tended to produce a stable foam in the extractor during the extraction, this foam being carried over into the extraction flask. A decrease in the sulfuric acid used for precipitation from 6 to 4 ml., and the phosphotungstic acid from 5 to 2.5 ml., yielded a serum which could be extracted with ether without difficulty. The polariscopic method of the A.O.A.C. (1) was used for lactose determination. In the case of skim milk and whey, correction for volume of the precipitate was made in accordance with the suggestion of Perkins (12).

RESULTS

Heating whole milk. In the first trials, whole milk, previously pasteurized at 62° C. and homogenized at 2500 pounds' pressure, was sealed in cans and then heated, either in a boiling water bath (100° C.) for various periods

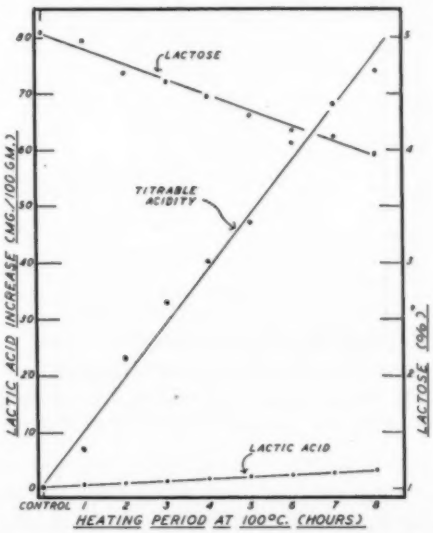

FIG. 1. Changes in titrable acidity, lactic acid and lactose when homogenized milk is heated in sealed cans at 100° C. (Titrable acidity expressed as mg. lactic acid/100 gm. milk.)

up to eight hours, or in the autoclave at 116° C. for periods up to 2.5 hours. The influence of these two heat treatments on the titrable acidity, lactic acid and lactose are shown in figures 1 and 2. These figures are nearly identical in that they reveal relatively small amounts of lactic acid formed (as measured by the Hillig method) in contrast to the amount of total acid formed. Figure 1 shows that heating the milk for eight hours at 100° C. resulted in an increase in titrable acidity equivalent to about 75 mg. of lactic acid per

100 gm., whereas the lactic acid increase was surprisingly small, amounting to about 3.5 mg. lactic acid per 100 gm. Heating milk at 116° C. for as long as 2.5 hours (figure 2) resulted in the total production of acid equivalent to 97 mg. lactic acid per 100 gm., whereas only about 5 mg. of true lactic acid was produced. Lactose concentration decreased from 5.05 to 3.95 per cent in the case of the 100° C. treatment for eight hours as contrasted to a change from 5.04 to 3.8 per cent for the 116° C.-2.5-hour treatment.

Heating skim milk. Skim milk was also heated at 100° C. for eight hours, but the data obtained are not presented since they were not appreci-

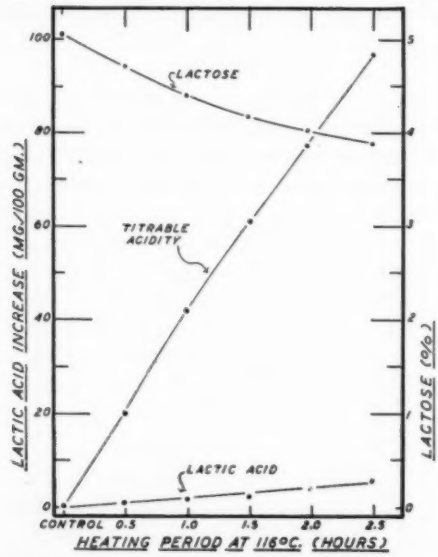

FIG. 2. Changes in titrable acidity, lactic acid and lactose when homogenized milk is heated in sealed cans at 116° C. (Titrable acidity expressed as mg. lactic acid/100 gm. milk.)

ably different from those obtained with whole milk. It was observed, however, that browning occurred more rapidly during heating with the skim milk than with the whole milk.

In other trials involving skim milk, attention was directed to the possibility that the relative proportion of lactic acid to total acid may be influenced by the addition of appreciable quantities of citrates or phosphates. With this in mind, 0.3 per cent of disodium phosphate or sodium citrate was added to skim milk and the milk then heated at 116° C. for 2.5 hours. Results of these two series are shown in figures 3 and 4.

In general, the trends for the various analyses are the same in these two figures and are not appreciably different than those shown in figure 2. It

may be observed that the presence of the two salts, in the concentrations used, appreciably increased the total acid production and the lactose destruction in comparison to the changes observed in the control samples, but only slightly altered the true lactic acid production. In the phosphate series (figure 3) the control samples showed an increase in lactic acid of about 4 mg. per 100 gm., whereas about 7 mg. per 100 gm. were produced in the phosphate-treated samples. In contrast, the difference in total acid production between the normal and phosphate-treated samples was equivalent to about 27 mg. lactic acid per 100 gm. Also, the lactose concentration in the

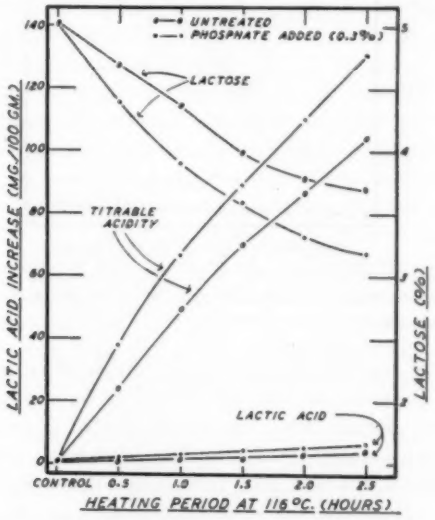

FIG. 3. Changes in titrable acidity, lactic acid and lactose when skim milk, either with or without added disodium phosphate, is heated at 116° C. (Titrable acidity expressed as mg. lactic acid/100 gm. milk.)

milk containing the added phosphate decreased appreciably more than in the control samples.

The citrate series (figure 4) varies from the phosphate only in so far as the magnitude of the differences between the control and salt-treated samples is concerned. The samples containing 0.3 per cent citrate underwent more browning, more total acid production, and more lactose destruction than the samples containing an equal quantity of phosphate. However, no appreciable difference in lactic acid production occurred. The greater effect by the citrate may be attributed to its slightly larger neutralizing power and thus its increased action upon lactose. However, the citrate ion produces other changes which may have a bearing on this particular case. One easily discernible effect of the citrate on milk is its clearing of the color. The

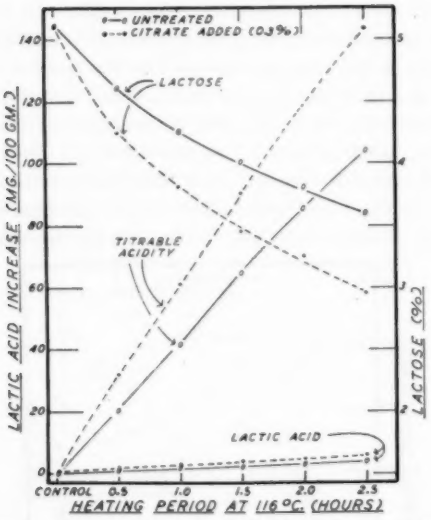

FIG. 4. Changes in titrable acidity, lactic acid and lactose when skim milk, either with or without added citrate, is heated at 116° C. (Titrable acidity expressed as mg. lactic acid/100 gm. milk.)

addition to milk of three-tenths per cent of citrate often noticeably reduced the opacity, and the milk gave the appearance of having been watered.

Heating whey. Rennin whey was heated at 116° C. for 2.5 hours and the results obtained on lactose, titrable acidity, and lactic acid (shown in figure 5). As a control, the skim milk from which this whey was obtained was likewise heated. However, the skim milk results are not presented since

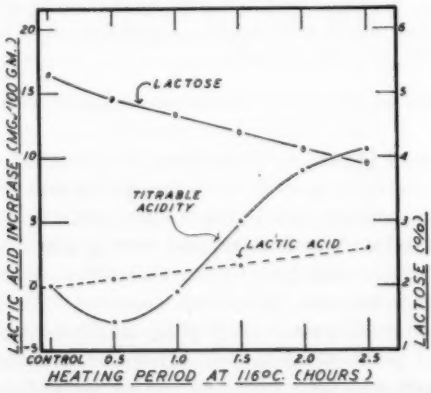

FIG. 5. Changes in titrable acidity, lactic acid and lactose when whey is heated at 116° C. (Titrable acidity expressed as mg. lactic acid/100 gm. whey.)

they were practically identical with the results for the normal samples shown in figures 3 and 4.

Figure 5 reveals that the heating of whey at 116° C. produced first a slight decrease in titrable acidity, followed by an increase, with a prolongation of the heating period. The decrease in total acidity at the 30-minute heating period, although slight, was observed in all three of the series conducted on whey, with the decrease varying to the equivalent of 2 to 5 mg. lactic acid per 100 gm. whey. At this point in the heating, coagulation in the whey proteins had occurred, but with no discernible browning. With further increases in the heating time, the acidity of the whey increased and the whey became brown in color.

The total acidity produced in the whey as measured by titration was approximately only one-ninth of that which was produced in the skim milk under similar heating conditions. As shown in figures 3 and 4, heating of normal skim milk for 2.5 hours at 116° C. produced the equivalent of about 104 mg. lactic acid per 100 gm. However, results with the whey show an increase of only about 11 mg. of acid per 100 gm. when expressed as lactic acid.

Although these marked differences in total acid formation in skim milk and whey occurred when these products were subjected to the same conditions of heat treatment, the lactose changes were practically identical. The loss in lactose in both cases amounted to approximately 26.4 per cent and 27.4 per cent of the original lactose content of the whey and skim milk respectively.

Figure 5 also shows that the increase in lactic acid is not greatly different than was observed in the skim milk, with the increases amounting to from 2 to 4 mg. lactic acid per 100 gm. whey for the 2.5 hours at 116° C. However, since the total amount of acid formed as measured by titration is so decidedly lower in the whey than in the skim milk, the relative proportion of this acid which is lactic is greater.

DISCUSSION

The results obtained in this study indicate that the amount of lactic acid formed when milk is heated constitutes a much smaller proportion of the total acid produced than has been previously reported. Even with the extreme conditions of temperature, time and salt adjustment used in these experiments, the lactic acid created was almost always less than 5 per cent of the total acid produced. This production of lactic acid by heat is much smaller than has been reported by Kometiani (10) and somewhat less than indicated by the work of Troy and Sharp (14).

Results obtained by Kometiani on heat-produced lactic acid are so high as to be questionable. Furthermore, they do not appear to be in line with the total acid (measured by titration) which he found to be produced by

subjecting the milk to the same heat treatment. Whereas the acidity increase as measured by titration was equivalent to about 75 mg. lactic acid per 100 gm. when the milk was heated at 120° C. for 30 minutes, the lactic acid determined quantitatively amounted to about 187 mg. per 100 gm. (assuming the specific gravity of the milk used was 1.032). This difference between increases in titrable acidity and lactic acid appears too great to be attributable to salt rearrangement and precipitation and does not show the agreement between the methods which one might expect on the basis of previous work (5). One factor to consider in this connection is the possible inaccuracy of the titration procedure used by Kometiani (10). This procedure, involving titration to the phenolphthalein end point, is of questionable accuracy when applied to milk which has become somewhat brown as a result of heat treatment.

The low lactic acid production by heat, as reported herein and as measured by the Hillig method, should add further evidence in support of a grading program utilizing lactic acid determinations as a basis of quality measurement. On a milk basis the heat processing ordinarily used in concentrated milk industries should result in formation of not more than 2–3 mg. lactic acid per 100 gm. if the assumption is made that the heat formation of lactic acid in a concentrated milk product proceeds at nearly the same rate as in the milk under the same conditions. However, even if lactic acid production by heat is influenced to some extent by the concentration of the product being heated, the total production by this means will likely be low under normal conditions. Thus, it would appear safe to conclude that, when a freshly manufactured concentrated milk product contains large quantities of lactic acid, the bulk of this acid may be attributed directly to the raw milk used.

In considering a study of a type herein presented, one should at all times be conscious that the data which are obtained are representative of certain selected methods, methods which have usually been developed for a certain determination in a normal product. However, if these methods are applied to a product whose "normalcy" has been affected by certain processes (in this case the effect of heat upon the milk), it is inconceivable to expect that the particular methods will always retain their accuracy or reliability. In fact, it should not be entirely unexpected to find at least certain of the methods not only to be unreliable when used on the processed product, but actually to be measuring other substances than that for which they were developed. As examples, the differences between the lactic acid finding in this and previous studies, and the failure to account for all of the lactose destruction by titration of the acidity, may both be at least partly accountable to the methods used.

Results presented in this paper dealing with the relationship between lactose destruction and titrable acidity are similar to those reported by

Whittier and Benton (17) and Kometiani (10) in that the lactose destruction is far more than enough to account for the acidity formed. In certain of the trials herein reported, increases in titrable acidity equivalent to about 104 mg. lactic acid per 100 gm. were accompanied by a decrease in lactose amounting to about 1300 mg. per 100 gm., or destruction of about 26 per cent of the total lactose present. On a molar basis, this lactose decrease was equivalent to a concentration of 0.036 as compared to an increase in the acid of 0.012, a ratio of 3 : 1. This ratio varied slightly with the different trials.

The question still arises as to whether this difference between lactose destruction and titrable acidity actually exists or whether it results from errors in the methods used which are created as a result of the heat treatment. The difference obtained may be the result of any one or more of the following: (a) the formation of certain non-acidic substances from the lactose, these substances not responding to titration and constituting a major portion of the breakdown products; (b) removal by the heating process of certain of the buffering agents in milk by salt precipitation, as suggested by Whittier and Benton (17), resulting in a lowered titrable acidity and an inaccurate measurement of the amount of acid formed; (c) inaccurate measurement of lactose, which presumably will indicate abnormally great lactose loss, the extent of this error being determined by the amount and type of degradation products which may influence either the copper-reducing capacity or the optical activity of the milk; (d) combination of a certain amount of lactose during heating with the proteins of milk, especially when browning occurs. This occurrence may also indicate abnormally large lactose destruction. Whatever the answer to this question, more experimentation is necessary before a complete solution is obtained.

It is interesting to compare the rate of acid formation obtained in these studies with values interpolated from the curve of Whittier and Benton (18). According to the curve, the rate of acid production at 116° C. is about 4.5 ml. N/10 acid per 100 ml. skim milk per hour. This is equivalent to 108.6 mg. of lactic acid per 100 gm. skim milk for 2.5 hours, and compares favorably with the 104 mg. lactic acid per 100 gm. obtained for the control samples in the citrate and phosphate series (figures 3, 4).

The results for the whey series reveal that the removal of the casein and the minerals which are associated therewith, greatly decreases the heat production of acid in the resulting serum. These results also show that a much greater amount of lactose is unaccounted for in the whey than in the skim milk. A full explanation of these observations must await additional information.

SUMMARY AND CONCLUSIONS

Whole milk and skim milk, heated in sealed cans at 100° C. for periods up to 8 hours, and at 116° C. for periods up to 2.5 hours, were examined for increases in titrable acidity by electrometric titration, lactic acid by essen-

tially the Hillig colorimetric method, and lactose by the polarimetric procedure. In certain trials, 0.3 per cent sodium citrate or disodium phosphate was added to the skim milk prior to heating.

The lactic acid produced by these treatments was within the ranges of 3 to 7 mg. per 100 gm. of milk and constituted only 5 per cent or less of the total acid produced as determined by titration. The presence of the stabilizing salts during heating appreciably increased the titrable acidity, but increased the lactic acid by not more than 2 to 3 mg. per 100 gm.

Lactose destruction, as a result of these heat treatments, amounted to about 25 to 30 per cent of the total lactose in the normal samples. Greater destruction occurred when the citrate or phosphate was present.

The slight increase in lactic acid as a result of heat treatment indicates that the use of proper lactic acid measurements on fresh concentrated milk products may be relied upon to reveal the quality of the raw milk used in their manufacture.

The heating of whey for 2.5 hours at 116° C. resulted in only about 11 per cent as much increase in titrable acidity as in skim milk, but losses in lactose and increases in lactic acid were similar.

The possibility exists that methods of analysis which may be acceptable for normal milk may not always yield reliable results when applied to milk which has been altered by high-temperature heat treatment.

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THE FORMATION OF VOLATILE ACIDS IN MILK BY HIGH-TEMPERATURE HEAT TREATMENT[1]

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In a previous publication (4) results are presented which reveal that the lactic acid formed when milk is heated to high temperatures will likely constitute not more than five per cent of the increase in titrable acidity, at least, when the lactic acid is determined by the Hillig colorimetric method (3, 6, 7). The problem, which immediately arises as a result of these findings, and concerning which there is little information, is the identification of the general characteristics, or perhaps the specific acids, which are responsible for the major portion of the heat-produced acidity. It is the purpose of this paper to present some results pertaining to this particular problem.

References to acids other than lactic, which are formed by heating milk, have been made by Leeds (9), Whittier and Benton (10) and Kometiani (8). In early work (1891) Leeds (9) indicated that the acid formed from lactose destruction was probably glucic. Whittier and Benton (10) reported that the distillate from heated milk possessed the aroma and gave certain tests for formic acid. Kometiani (8) found formic acid to represent about 20–25 per cent of the total lactose loss. The amount of formic acid produced was about 22 mg. per 100 ml. milk when the milk was heated at 100° C. for 3 hours, or 42 mg. per 100 ml. milk when the heating was at 120° C. for 30 minutes. According to Kometiani (8) the amount of lactic acid is four to five times the amount of formic acid, and the two acids together account for the total loss in lactose.

Since the previous studies by the author have indicated the rather insignificant rôle lactic acid (a non-volatile acid) plays in connection with the production of acid by heat (4), it appeared desirable to give attention to the volatile acids which may be involved. Consequently, trials were conducted with the view of (a) ascertaining the influence of heat upon the volatile acidity of milk, and (b) determining to some extent the principal volatile acid present.

PROCEDURE

Either homogenized whole milk or freshly separated skim milk was used in these trials. However, since the results for the homogenized milk did not vary appreciably from those of the skim milk, the skim milk data only are

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presented. The milk was sealed in "Canco" No. 2 cans and heated in the autoclave at 116° C. for the specified periods. Details of the heating procedure are described in the earlier paper (4).

Steam distillation of the skim milk was conducted by using the multiple unit apparatus described previously (5). Two hundred fifty ml. of the sample was placed in a one-liter, long-necked, round-bottom flask, brought to about pH 2 with 40 ml. N H_2SO_4, and the volume then adjusted to 300 ml. A small quantity of paraffin was added to prevent foaming, and the sample then steam-distilled at the rate of approximately 300 ml. per hour.

The sample was maintained at constant volume during distillation by proper temperature control. Distillation was continued until 600 ml. of distillate was obtained. The distillate was collected in 100-ml. volumetric flasks, transferred with several rinsings of water to a beaker, and then titrated to the phenolphthalein end point with N/20 NaOH. Each sample was corrected for a blank amounting to 0.25 ml. N/20 NaOH, obtained by titrating a sample consisting of 100 ml. of distillate from previously distilled water plus the volume of water used for the rinsings.

In one portion of the study the steam distillate obtained from the skim milk was concentrated and redistilled. This was accomplished by combining the distillate (following titration) from duplicate samples of skim milk into a three-liter, round-bottom flask. At this point the total volume of liquid was approximately 2,000 ml. (consisting of 600 ml. of distillate from each sample plus the alkali used for titration and the water necessary to make the transfers). To make certain that the acids were completely in the form of their salts and, thus, for all practical purposes would be non-volatile, an additional 2 ml. N NaOH was added. The solution was then concentrated to a volume of about 50 ml. by boiling under vacuum, the boiling temperature being about 60° C.

The concentrated distillate was redistilled under conditions similar to those used for the skim milk, the principal differences being that only 7 ml. of N H_2SO_4 was added to adjust the pH to about 2, that the volume was brought to 200 ml. instead of 300 ml., and that no paraffin was necessary.

Pure acids were steam distilled under the same conditions as were used for the concentrated distillate.

Determination of formic acid was made essentially by the AOAC method (1) and was conducted on the acidified concentrated distillate from skim milk. One-hundred-ml. samples were used instead of the recommended 50-ml. samples.

RESULTS

Volatile acidity of heated skim milk. In the first series of trials, raw skim milk and skim milk heated for one and two hours at 116° C. were steam-distilled. Results shown in figure 1 reveal that heating at three temperatures and times definitely increased the production of volatile acids. The

raw control sample of skim milk exhibited a slight amount of volatile acidity, the 600 ml. of distillate requiring an average of 3.3 ml. N/20 NaOH. In contrast, the same volume of distillate from the samples heated for one- and two-hour periods required about 13 and 24 ml. of N/20 NaOH respectively.

This figure also reveals that considerable amounts of volatile acids are being evolved at the close of the distillation period. The first 100 ml. portion always possessed the highest titer of any similar volume of distillate, but thereafter the acids were volatilized at a fairly uniform rate. Continuation of the distillation for a longer period of time would doubtless have resulted in an eventual decrease in the rate at which the acids were evolved. However, the use of prolonged periods of distillation was deemed inadvisable due

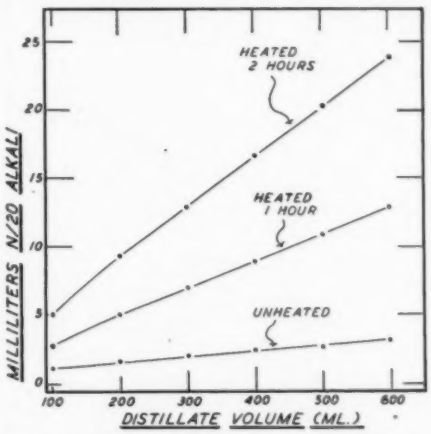

FIG. 1. Effect of heating milk at 116° C. for one- and two-hour periods on the steam volatile acidity.

to the possibility of having additional acids formed at the temperature at which the distillation was conducted.

Distillation of skim milk distillate. Since volatile acids were found to result from the heat treatment, trials were conducted with the view of gaining some knowledge of the volatility of the acids involved when they were free from the constituents of milk. To accomplish this, the steam distillate from skim milk was obtained, concentrated and then redistilled under controlled conditions. In order to make a comparison of the rate of distillations, pure formic and acetic acids were also distilled under the same conditions. These acids were used in concentrations comparable to those of the acids in the skim milk distillate, *i.e.*, concentrations equivalent to about 40–45 ml. N/20 NaOH. Figure 2 shows the distillation results obtained for formic and acetic acids and for the skim milk distillate. Included in this figure also are results given by Dyer (2) for the distillation rate of formic and acetic acids.

This figure shows that the rates of distillation of the pure acids as obtained in this study are similar to, but slightly lower, than those obtained by Dyer (2). These slightly lower results may be attributed to the fact that (a) a larger volume of sample was used in this study, and thus a slightly greater amount of the acid may be retained in the sample flask; and (b) that a correction for a blank was made on each titration, a correction not indicated by Dyer (2). Irrespective of these slight differences, the results show distinctly the comparable rates at which formic and acetic acids are steam-

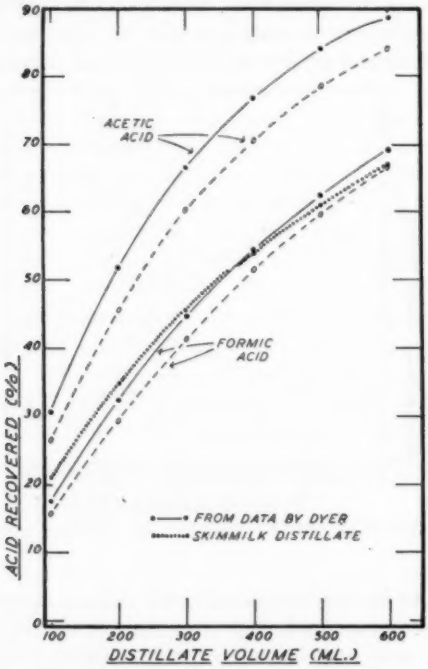

FIG. 2. Recovery of formic and acetic acids and the acids in skim milk distillate by steam distillation.

distilled. In this study the distillation constant (per cent of acid distilled over in first 100-ml. portion), was 26.5 for acetic and 15.8 for formic. Distillation constants reported by Dyer (2) were 30.75 and 17.89 for acetic and formic acids respectively.

The distillation rate of the skim milk distillate is calculated on the basis of 100 per cent for the total titer of the distillate obtained from the skim milk. The rate of distillation of the skim milk distillate is approximately five per cent greater than the formic acid rate in the first portions, but tends to become closer as the distillation proceeds. In general, the curve for the

distillate may be said to follow fairly closely that of the formic acid. Approximately 67 per cent of the total volatile acids was recovered in the 600 ml. of distillate.

Formic acid determination. The similarity between the formic acid curve and the skim milk distillate curve, as shown in figure 2, indicates that formic acid may be an important constituent of the volatile acids formed when milk is heated. To ascertain if such were the case, samples of the concentrated skim milk distillate were analyzed for formic acid. However, before using the skim milk distillate for formic acid determinations, the AOAC distillation method was subjected to some attention to ascertain its ability to recover formic acid from an aqueous solution when the volume of the sample was maintained at 100 ml. during distillation. Results of eight trials are presented in table 1.

TABLE 1

Recovery of known amounts of formic acid from 100 ml. of aqueous solution

Trial No.	Formic acid added (Milligrams)	Formic acid recovered				
		Milligrams		Per cent		
		(a)	(b)	(a)	(b)	Ave.
1	175.0	137.8	131.9	78.7	75.4	77.0
2	87.5	71.6	74.3	81.8	84.9	83.3
3	87.5	74.9	73.8	85.6	84.3	84.9
4	87.5	67.5	71.8	77.1	82.0	79.5
5	87.1	66.6	66.8	76.5	76.7	76.6
6	87.1	74.0	71.7	84.9	82.3	83.6
7	98.7	77.4	78.0	78.4	79.0	78.7
8	98.7	.....	77.2	.....	78.2	78.2
Average per cent recovery for eight trials						80.2

These data show that the method as used recovers, on the average, approximately 80 per cent of the formic acid added, with the recovery for the individual determinations varying within the extreme ranges of about ± 5 per cent from this average. Five of the eight duplicate averages were within 3.5 per cent of the 80.2 per cent average.

From these observations, it appeared that this method of measuring formic acid could be applied to the milk distillate with a fair degree of accuracy provided proper correction was made to account for that portion of the acid retained by the sample. Consequently, determinations were conducted on acidified samples of skim milk distillate, and the results obtained were corrected on the basis that they represented only 80 per cent of the total formic acid present. Results are presented in table 2 for unheated and heated samples of milk.

The purpose of analyzing the distillate from the raw skim milk was to ascertain if the steam distillation process itself resulted in formic acid pro-

duction. The data obtained do indicate a small quantity of formic acid in these samples, the amount varying from 1.2 to 3.2 mg. Since the total titers of the distillates from the raw skim milk were low, small variations in the amount of formic acid obtained resulted in rather large variations when expressed as the percentage of the total, *i.e.*, variations from 9.8 to 24.2 per cent. However, when consideration is given to the data obtained with the heated samples, the amount of formic acid resulting from raw milk being subjected to the steam distillation process is of no great significance.

A comparison of the formic acid data for the raw and heated samples reveals the marked influence of the heat treatment at 116° C. on formic acid

TABLE 2

Formic acid obtained from steam distillate of raw skim milk and skim milk heated at 116° C. for two hours (steam distillate represents 1200 ml. distillate from 500 ml. milk)

Sample	Total titer of distillate expressed as formic acid (a)	Formic acid recovered		Proportion of total acid (a) which is formic (e)
		Actual amount (b)	Corrected* amount (c)	
	mg.	*mg.*	*mg.*	%
Raw	13.2	2.6	3.2	24.2
Raw	12.2	1.0	1.2	9.8
Raw	13.6	1.6	2.0	14.7
Heated	82.9	55.5	69.4	83.7
Heated	77.2	52.3	65.4	84.7
Heated	80.7	54.4	68.0	84.3
Heated	80.0	54.1	67.6	84.5
Heated	94.5	63.1	78.9	83.5
Heated	85.6	55.1	68.9	80.5
Heated	83.4	56.9	71.1	85.2

* Correction is made on basis that amount of formic acid obtained by the method used represents only 80 per cent of the total in the distillate sample.

production. The amount of formic acid obtained from the distillate (when corrected for the retention factor) ranged from about 65 to 79 mg. and represents 80–85 per cent of the total volatile acidity. The proportion of total volatile acid found to be formic averaged 83.8 per cent for the seven trials with the heated milk.

DISCUSSION

Although, in the study herein presented, rather extreme heat treatments of milk are used to demonstrate the production of volatile acids, nevertheless it may be expected that these acids (in lesser quantities) may be produced when lower temperatures and shorter holding periods are utilized. Presumably, the heat treatment must be sufficient to cause some lactose destruction before these acids (particularly formic) occur. The fact remains, however, that in those processes in the dairy field utilizing high temperatures, certain quantities of these volatile acids may be produced. Furthermore,

the presence of these acids, even in extremely small concentrations, may appreciably affect the quality of the particular product involved.

An important consideration in regard to the formic acid determinations is that the results obtained do not represent the total formic acid in the original milk sample. As shown by figure 1, the volatile acids are being distilled from the milk in appreciable quantities, even at the close of the 2-hour distillation period. Therefore, a prolongation of the distillation period would have resulted in greater quantities of volatile acids in the distillate and thus an increase in the quantity of formic acid. It is also possible that, with a prolonged period of distillation of the milk, the proportion of formic acid to total acid may be altered.

Even though only a small quantity of formic acid was found in the distillate from raw milk and may be the result of a small amount of decomposition of the milk during steam distillation, it does not necessarily result that the same degree of effect will occur with a heated sample. A heated sample of milk in which considerable change in acid production and lactose destruction has occurred may undergo even more changes as it is subjected to the high temperature used for steam distillation.

CONCLUSIONS

Heating skim milk in cans at 116° C. for one- and two-hour periods appreciably increased the volatile acid content of the milk as determined by steam distillation.

Redistillation of steam distillate obtained from skim milk heated previously for two hours at 116° C. resulted in a curve falling between the formic and acetic acid curves at the start of the distillation, but resembling that obtained from formic acid for the major portion of the distillation period. Under the conditions of this experiment, formic acid constituted 80–85 per cent of the total volatile acids in the skim milk distillate.

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SOME RELATIONSHIPS BETWEEN pH, TITRABLE ACIDITY, AND THE FORMOL TITRATION IN MILK HEATED TO HIGH TEMPERATURES[1]

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In previous papers (1, 2) results are presented of studies pertaining to the influence of high temperature heat treatment on titrable acidity, lactic acid formation, volatile acidity, and formic acid production. At the time these previous studies were conducted, attention was directed to the changes in pH and in the formol titration of milk, skim milk and whey as created by the heat processing and as influenced by phosphates, citrates, and oxalate. Results of these observations and the relationship between certain of the findings are the basis for this paper.

In the review of literature presented previously, dealing with heat-produced acidity (1), it is pointed out that the lactose is usually considered the major, if not the entire, source of the acid formed. Furthermore, the comparatively large quantity of formic acid (an acid derived from sugars and not from proteins), which is produced as the result of heat (2), also indicates that the lactose plays an important rôle in this connection. However, the possibility has not been entirely eliminated that certain protein changes also may result from the heat treatment and that these protein changes may directly or indirectly affect the values obtained by titration of the milk with an alkali. Presumably, if protein changes occur involving rearrangement or production of carboxyl groups, they may be detected by application of the formol titration procedure. Limited and somewhat contradictory data along this line have been presented by Kometiani (4) and Kass and Palmer (3).

Kometiani (4) expresses the belief that the chemical nature of the casein molecule is affected by heat, resulting in an increase in free carboxyl groups and in acidity, but that the acidity from this source is quantitatively considerably smaller than that which occurs from lactose decomposition.

To determine the effect of heat on the proteins, Kometiani (4) heated milk to 80°, 100°, 110°, and 120° C. and determined the increase in formol titration by titrating milk to the phenolphthalein end-point before and after treatment with formalin. The following increases in formol titration were obtained (expressed as ml. N/10 NaOH per 100 ml. milk) for the different temperatures: 80° C.—0.0; 100° C.—0.03; 110° C.—1.9; 120° C.—3.2. Kometiani (4) indicates that it was not easy to obtain uniform end-points in titrating the milk which has browned by heat.

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[2] Now at the University of Maryland, College Park, Md.

Kass and Palmer (3) present in abstract form the results of a study in which formol titrations were made electrometrically on artificial, skim, and dialyzed milks before and after autoclaving for 15 minutes at 120° C. This study was conducted "on the assumption that a heat-induced combination between lactose and casein at the amino groups should result in an increase in the titrable acidity exhibited before the formaldehyde is added in the Sorensen procedure, since the condensation of the sugar with the amino groups should mask the basicity of the latter and cause an increase in the free carboxyl groups capable of neutralization. Furthermore, such a condensation should cause the pH of the protein sol to be higher than normal when the formaldehyde is added to the initially neutralized sol, and the subsequent true 'formol titer' will therefore be correspondingly low."

Although they present no specific data, Kass and Palmer (3) report that the formol titer of skim milk was slightly increased by heating at 120° C. for 15 minutes, but that this effect was obliterated by the addition of potassium oxalate solution. The addition of the oxalate intensified the difference in the initial titers of raw and autoclaved milk, but only slightly affected the formol titers. Milk dialyzed free of lactose gave results similar to those obtained on skim milk from the standpoint of the formol titration, with the slight increase resulting from heat being completely overcome by oxalate treatment.

On the basis of their results, Kass and Palmer (3) question the validity of the formol titration of milk "in seeking evidence for an aldose-casein condensation."

That other factors, in addition to the protein, affect the formol titration has been demonstrated by Pyne (6). This worker believes that the formaldehyde titration gives a composite result, dependent principally on the protein of the milk, but also influenced by the phosphates. He proposes the use of oxalate to eliminate the phosphate effect. Kass and Palmer (3) found that the non-protein factors influencing the formol titration were not eliminated by oxalation.

PROCEDURE

Specific information relative to the milk used and to the heating procedure is given in previous papers (1, 2). Both whole milk (previously pasteurized at 143°—30 minutes and then homogenized) and skim milk were heated in cans in a boiling water bath (100° C.) or in an autoclave (116° C.). Cans were removed at intervals to permit examination of the milk. In certain of the skim milk samples, either 0.3 per cent disodium phosphate or sodium citrate was dissolved in the milk prior to the heat treatment.

Titrations were conducted electrometrically, using a Beckman type-G pH meter, a type-E glass electrode. Fifty-ml. samples were used for the titrations, the samples being stirred vigorously during titration by a motor-driven agitator. Neutralization was accomplished with N/10 NaOH, and pH 8.3 was established as the end point for the titrations.

Formol titration was conducted by neutralizing a 50-ml. sample of milk to pH 8.3, then adding 5 ml. formalin (previously adjusted to pH 8.3) and retitrating to pH 8.3. The amount of formalin used is in accordance with the suggestion of Levy (5) that the concentration of formalin in the final volume after titration should be between 6 and 9 per cent.

The oxalate treatment was conducted by adding 2 ml. of a neutral saturated solution of potassium oxalate to the milk and allowing the sample to mix thoroughly for about two minutes prior to titration.

Whey was obtained by rennet coagulation of skim milk followed by straining to remove the coagulum.

The various treatments are indicated as follows:

Normal—Untreated sample.

Oxalate—Sample treated with oxalate prior to titration.

Formol—Formol titration, *i.e.*, addition of formalin to sample following electrometric titration and then reneutralizing to pH 8.3.

Formol oxalate—Formol titration of a sample treated with oxalate.

RESULTS

Heating whole milk. Results obtained by heating whole homogenized milk at 100° C. for periods up to eight hours are shown in figures 1 and 2. In figure 1 are shown the increases in acidity produced by the heat treat-

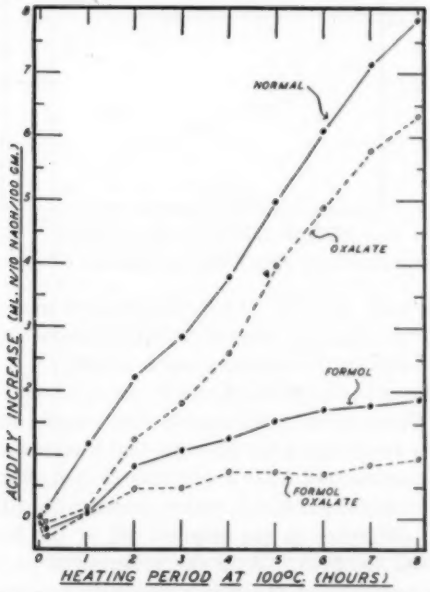

FIG. 1. Acidity changes produced when whole homogenized milk is heated at 100° C.

ment, and in figure 2 are shown the pH changes. In the case of acid production it may be observed that increases occur in each of the curves. The oxalate curve practically parallels the curve for the titrable acidity of the normal milk, with the exception that a marked delay in acid increase at the 0.5-hour period is indicated. The uniformly lower titers for the oxalate samples may be expected in view of the influence of the oxalate on the calcium salts.

An increase in the formol titration with prolongation of the heating period also is indicated by figure 1, the increase being insignificant at the end of the first one-hour period but gradually increasing thereafter. This increase in the formol titration was markedly reduced but not entirely obliterated by the addition of oxalate.

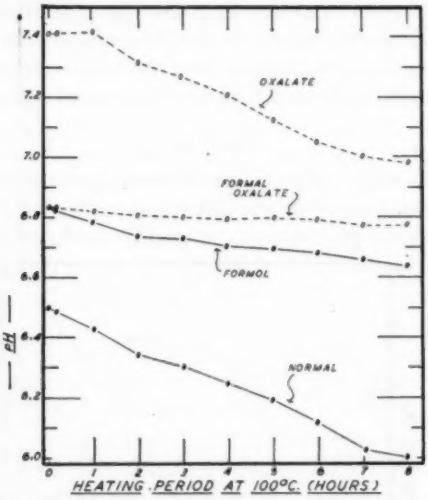

FIG. 2. pH changes produced when whole homogenized milk is heated at 100° C.

Heating of the milk for only 10 minutes resulted in a slight lowering of the oxalate, the formol, and the formol-oxalate titration, although this lowering was not observable in the normal titrable acidity.

The pH changes in the samples (figure 2) are in direct relationship to the acidity changes. The original milk treated with oxalate had an original pH of 7.4, but the same treatment after the milk had been heated for eight hours gave a pH of approximately 6.97, a decrease in pH of 0.43. The control milk possessed an original pH of 6.5, which decreased to pH 6.0, a change of 0.5 pH unit. No difference in the original pH of the formol and formol-oxalate samples was observed. With the progression of the heating, however, the pH values of the formol samples decreased to a greater degree than those of the formol-oxalate samples.

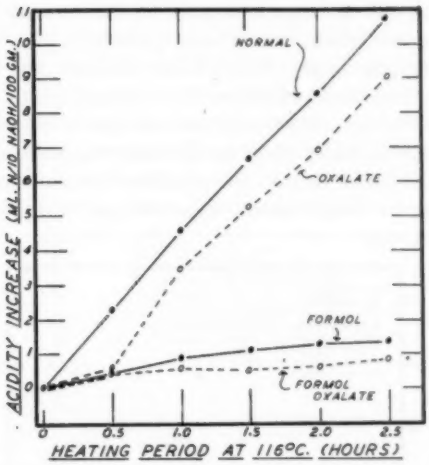

FIG. 3. Acidity changes produced when whole homogenized milk is heated at 116° C.

Heating whole milk at 116° C. for 2.5 hours produced changes in acidity and pH comparable in trend to those produced at 100° C. These results are

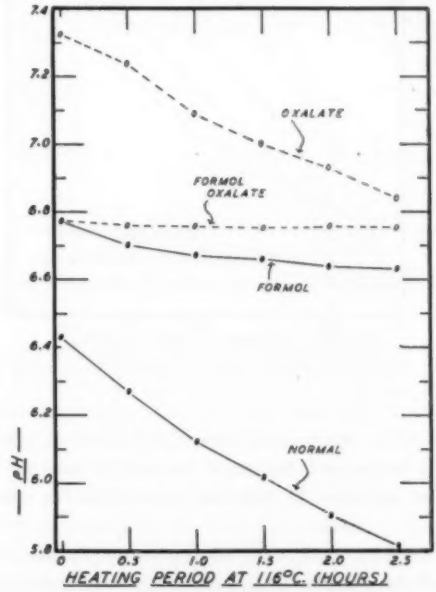

FIG. 4. pH changes produced when whole homogenized milk is heated at 116° C.

shown in figures 3 and 4. Again, it may be observed in figure 3 that the oxalate curve is lower than, and almost parallel with, the normal curve throughout the heating range. Thus, it may be said that the oxalate treatment reduces the magnitude of the acidity changes produced when milk is heated, and the difference between the normal and oxalate curves indicates the extent of the calcium salt effect on the heat-produced acidity. Figure 3 is also similar to figure 1 in that the oxalate treatment again reduced the extent of the formol titration changes but did not completely overcome the heat-produced increase.

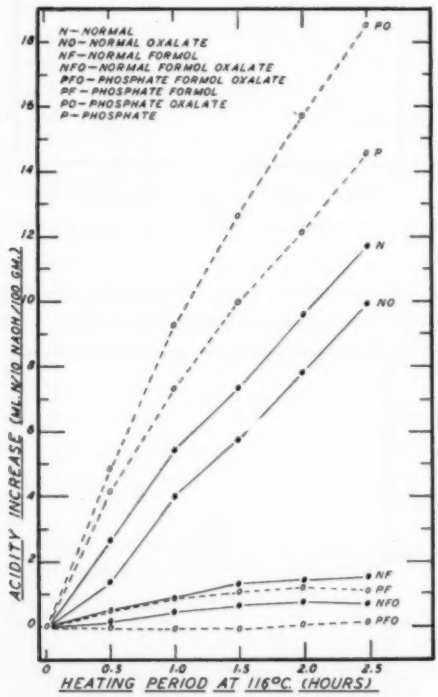

FIG. 5. Acidity changes produced when skim milk containing 0.3 per cent added phosphate is heated at 116° C.

Heating skim milk. Skim milk was also heated at 100° C. for varying periods up to eight hours and examined for acidity and pH changes. Since the results were not appreciably different than those for whole milk, they are not presented.

In other trials, skim milk with and without disodium phosphate (0.3 per cent), or sodium citrate (0.3 per cent) was heated at 116° C. for periods up to 2.5 hours. Results of the phosphate series are presented in figures 5 and 6.

The acidity effects in figure 5 reveal a similar relationship between the various curves of the normal milk as was observed in figures 1 and 3 for whole milk; *i.e.*, the oxalate reduced the normal acidity changes and the formol titration. In the case of the phosphate-treated samples, however, different results were obtained. In these trials, the treatment of the milk with oxalate intensified the effect of the heat rather than decreasing it as in the case of normal milk. This intensification of the effect of heat was more pronounced as the heating period progressed, with the phosphate oxalate

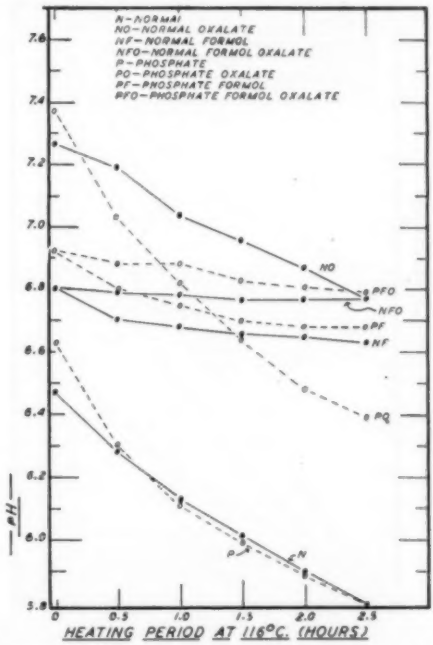

FIG. 6. pH changes produced when skim milk containing 0.3 per cent added phosphate is heated at 116° C.

curve exceeding the phosphate curve by approximately 28 per cent at the 2.5-hour period.

Another interesting observation is that, although the phosphate-treated samples underwent more acidity production than the normal sample, the phosphate-formol titration was not higher, but slightly lower, than that of the normal-formol. Furthermore, whereas the addition of oxalate partially reduced the extent of change in the formol titration of normal milk, it completely obliterated such changes in the phosphate treated milk.

The pH curves (figure 6) show the greater degree of change which occurs in the phosphate samples. For example, the original pH of the phosphate-

oxalate sample was higher than that for the normal-oxalate sample, but underwent considerably greater change upon heating. A somewhat similar relationship is shown between the other comparable curves. Also revealed in this figure, and in figures 2 and 4, is that in the formol curves the original pH was the same irrespective as to whether or not the oxalate had been added. Furthermore, the starting pH for the formol curves is uniformly at a slightly higher level than for the original milk, and consequently somewhat less alkali is required to readjust the pH to 8.3.

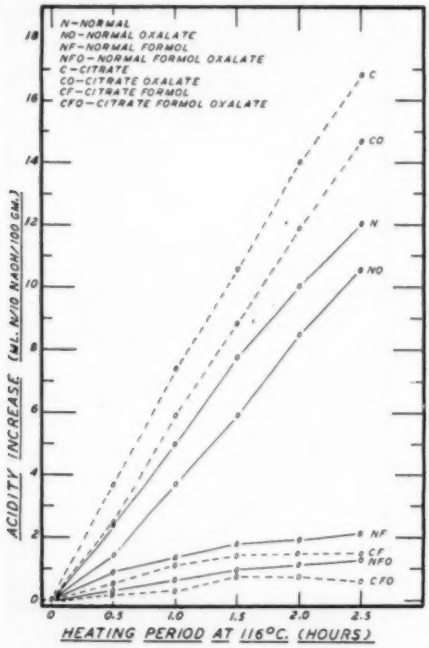

FIG. 7. Acidity changes produced when skim milk containing 0.3 per cent added citrate is heated at 116° C.

A striking difference between the phosphate and normal series is that the phosphate samples do not show a normal relationship between acidity changes and pH. For example, the pH values of the phosphate samples after heating are decidedly lower than for the phosphate oxalate samples. Thus, one might expect that less alkali would be required to bring the phosphate oxalate samples to pH 8.3. However, the reverse it true. In the case of the normal series, however, a lower pH is directly related to a higher titer.

The heating of milk containing added citrate resulted in changes in acidity and pH that differ somewhat from those obtained with phosphate

(figures 7 and 8). The oxalate treatment produced acidity changes similar to those obtained in the untreated milk, with the citrate oxalate curve persisting at a lower level than the citrate curve for the various heating periods. In the case of the formol results, the citrate-formol-oxalate curve resembles the phosphate-formol-oxalate curve in that the oxalate treatment practically eliminates the effect of heat on the formol titration.

Figure 7 also shows that, even though the citrate sample underwent more acidity production upon heating than the untreated sample, the respective

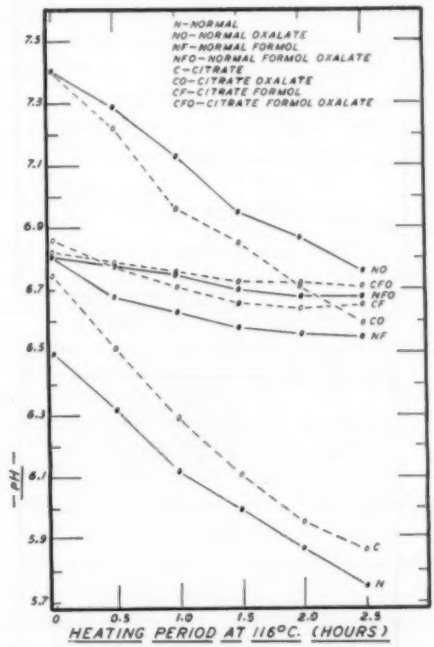

FIG. 8. pH changes produced when skim milk containing 0.3 per cent added citrate is heated at 116° C.

formol titrations were not greatly different. In fact, the citrate formol curve persisted at a slightly lower level than the normal formol curve throughout the heating period, although the difference is so slight as to be of no great significance. In this respect, then, the citrate resembles the phosphate curve.

pH changes in the citrate series (figure 8) are entirely as might be expected from titration results.

Heating of whey. The heating of whey resulted in formol titration curves somewhat different than those of milk or skim milk, as may be observed in figure 9. Not only was the heat effect on the formol titrations of

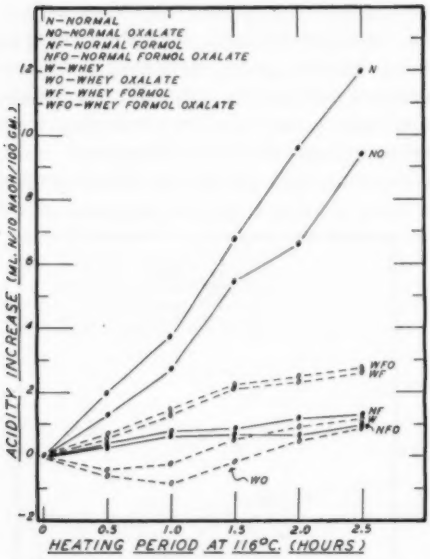

FIG. 9. Acidity changes produced when whey is heated at 116° C.

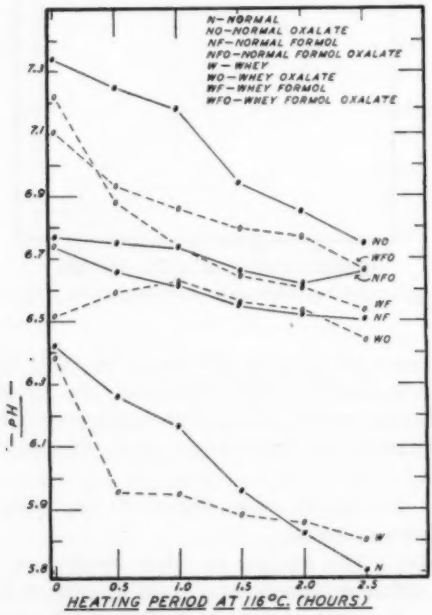

FIG. 10. pH changes produced when whey is heated at 116° C.

whey distinctly greater than for skim milk, but the effect was practically unaltered by oxalate treatment. This contrasts to the formol titer for skim milk (normal), which was distinctly reduced by the oxalate treatment.

Figure 9 also reveals the low acid increase which results from heating whey, an observation which was also noted in a previous paper (1). In fact, heating of the whey for periods of 0.5 and one hour reduced, rather than increased, the titer. This reduction was intensified by the oxalate treatment.

The pH curves for whey (figure 10) do not, in all cases, correlate closely with the titration results. For example, the whey pH curve shows a marked drop at the 0.5-hour period, whereas the whey actually required less alkali to adjust the whey to pH 8.3. In contrast, the whey oxalate pH curve follows the trend expected on the basis of the titration curve.

Figure 10 also reveals that the pH of whey following the addition of formalin is markedly above the pH of the skim milk following similar treatment.

DISCUSSION

Results obtained in this study permit the drawing of several interesting observations in regard to the formol titrations. The data do reveal an increase in the formol titration when milk is heated at high temperatures, but it would appear that this increase is extremely slight at those temperatures and times which may be used under commercial conditions. The formol titration does not appear to be closely allied with browning of milk, since there is no abrupt change in this value at or about the period at which browning occurs. Instead, the formol titration appears to increase at a slow rather uniform rate throughout the heating process.

The data also indicate that the salts of milk are responsible for an appreciable portion of the formol titration increases, since the treatment of the heated milk with oxalate greatly reduced the increase in titer noted. Furthermore, the failure of the oxalate treatment of whey to decrease the formol titration may indicate that the salts involved are associated with, and removed with, the casein. In contrast is the fact that the oxalate treatment of the milk containing the added phosphate completely obliterated the heat effect on the formol titration.

In connection with the formol titration, however, it should be pointed out that there is no indication that the presence of added phosphate would in itself appreciably affect the titer. The average formol titrations for three trials of milk was 17.01 ml. N/10 NaOH per 100 gm., whereas the same milk containing 0.3 per cent added phosphate had an average formol titer of 17.4 ml. N/10 NaOH per 100 gm. Treatment of these two lots of milk with oxalate resulted in a formol titer of 18.51 ml. N/10 NaOH per 100 gm. for the untreated milk and 18.43 ml. N/10 NaOH per 100 gm. for the milk containing the phosphate.

Although sodium dihydrogen phosphate and sodium citrate are both

considered in the heat stabilization of milk, they show somewhat different properties in so far as certain of the determinations used in this study are concerned. Of particular interest is the fact that oxalate treatment of heated milk containing the phosphate increased the titer, whereas the reverse was true in the untreated milk and in the milk containing added citrate. This increase in titer in the case of the phosphate series may be the result of an increase in the buffer capacity of the milk created upon the addition of the oxalate. Such an occurrence may logically result through conditions similar to those described by Sommer and Menos (7), in which the precipitation of tricalcium phosphate permits dissociation of the tertiary phosphate, thus increasing the amount of alkali necessary for neutralization. In the case of the results herein presented, the oxalate precipitation of the calcium in the presence of an excess of phosphate may also permit a dissociation of the tertiary phosphate iron, which may not normally occur.

The oxalate curves for heated samples of normal milk differ from the normal curves principally in the fact that they reveal a definite retardation of acidity during the first period of heating. Thereafter, the slope of the oxalate curve is less than, but almost parallel with, that of the normal curve. This divergency of the oxalate curve from the normal curve during the first period of heating is especially revealed by figures 1 and 3, where the heating was at 100° C., but is also shown, but to a less degree, in figures 5 and 7 where the heating temperature was 116° C. These results may indicate that the first acidity increases in milk produced by heat and measured by titration are due, not to true acid production, but rather to salt rearrangements which increase the buffer capacity of the milk. In this connection it should be pointed out that the divergence of the oxalate from the normal curve during the first stages of heating is not so apparent when the milk contains added phosphate (figure 5).

SUMMARY AND CONCLUSIONS

Whole homogenized milk or skim milk heated at 100° C. for periods up to eight hours, and at 116° C. for periods up to 2.5 hours, was examined for acidity changes before and after adding oxalate, for formol titration changes before and after adding oxalate, and for pH changes. Sodium citrate (0.3 per cent) and disodium phosphate (0.3 per cent) were added to the skim milk in certain of the trials. Titrations were conducted electrometrically.

Acidity changes produced by the heat treatments were reduced when the milk was treated with oxalate prior to titration in all milk excepting that containing added phosphate. The oxalate treatment of the phosphate milk, however, resulted in higher titers, the difference being more marked as the heating period progressed.

The heating of milk under the conditions of these experiments resulted in a slight, but definite, increase in the formol titration. This increase was appreciably reduced in the normal samples upon the addition of oxalate, whereas in the phosphate samples it was completely obliterated.

pH changes correlated well with the majority, but not with all of the titrations. Changes in the buffer capacity of milk, as produced either by the addition of salts or through heat may influence the titration without a similar influence on the pH.

Heating of whey at 116° C. produced changes which varied from those produced by heating milk. The whey exhibited greater increases in formol titration than did the skim milk from which it was obtained, and the formol titration increase was unaffected by treatment of the whey with oxalate. This may indicate that the salts which are responsible for an important portion of the formol titration increase are associated with, and removed with, the casein in the preparation of the whey.

The comparative slopes of the acidity increase-heating time curves for normal- and oxalate-treated milk reveal that the first acidity increase in milk as detected by titration may be due to salt changes, resulting in an increase in the buffer capacity.

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A 1.10–PHENANTHROLINE METHOD FOR THE DETERMINATION OF IRON IN POWDERED MILK

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Milk contains traces of iron. The amount reported in the literature varies over a very wide range. Stugart (14), Associates of Rogers (2), and Davies (5) summarize the data available on the iron content of milk and state that the amount varies from 0.21 to 56.8 parts per million. Sherman (12) in 1941 reported the iron content of milk as two parts per million. More recently Johnston (7), using Stugart's thiocyanate method (14), analyzed sixty-one bottles of market milk from twenty-five companies in twelve communities. The iron content ranged from 0.114 to 0.650 milligrams per kilogram with a mean of 0.319 mg. per kg. This author also brings out the fact that, during the past ten years, seven out of eight investigations obtained values from 0.34 to 0.96 mg. per kg. Johnston concludes that the best value to adopt for average market milk is 0.3 mg. per kg., and that this value is one-seventh of the value given in previously reported tables of food composition.

The wide variation in the results obtained for the iron content of milk can be traced, no doubt, not only to variable degrees of contamination of the milk but to the variable methods of analysis employed and to the different procedures used in preparing the sample for analysis.

It is to be expected that the equipment used in handling milk at the farm and in the plant will result in a certain amount of metal contamination. It has long been recognized that, in order to prevent or retard the development of off flavors, especially of the oxidized type, the iron content of milk and other dairy products must be kept to a minimum. In recognition of this fact, the Army Quartermaster Corps has placed a maximum of 10 parts per million of iron in their buying standards for powdered whole milk, a standard which in view of the findings of Johnston on the iron content of milk seems very liberal.

There are numerous methods that have been used for the determination of iron in biological materials. Until recently most of these determinations were made either volumetrically, gravimetrically, or by visual means. It is now recognized that the photoelectric spectrophotometer provides a means of quantitative analysis that is of a higher degree of accuray than is possible by other methods. This is particularly true when there are only traces of the metal present.

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Since the determination of traces of iron in dried milk and milk products is so important, it was thought advisable to study the adaptability of spectrophotometer methods to this problem, with the idea of developing a rapid, accurate test not too complicated in its procedure.

PREPARATION OF THE SAMPLE

The sample may be prepared for analysis by either the wet or dry ashing methods. The wet digestion procedure was successfully used on a few samples but was found to be less convenient than the dry ashing method. When the wet digestion method is used, acid must be added every few minutes during the digestion period; and the samples must be more or less constantly watched throughout the digestion. In some cases a white insoluble precipitate forms which is likely to detract from the accuracy of the analysis. It is also essential that the laboratory be equipped with an effective means of removing gas fumes issuing from the sample while it is being digested.

In the dry ashing procedure it is necessary to eliminate interference from pyrophosphates. This can be done either by fusion with sodium carbonate or by the addition of an acid such as hydrochloric or nitric. When the sample is prepared using sodium carbonate fusion, it is necessary to transfer the sample from the dish to the mortar and, after grinding, transfer it back again to the same dish. The ash is light and feathery, and with the best of care some of the fine ash particles may be lost. Sodium carbonate, even though it is purified before adding, may be a source of contamination. The mortar and pestle may also be a source of contamination. Early in this investigation it was found that results on iron using sodium carbonate fusion were much lower than when the dry ash was hydrolyzed with hydrochloric acid. Further work showed that the length of time of fusing had a definite bearing on the results obtained. In the literature none of the methods using sodium carbonate fusion specified the length of fusion time. It is evident, however, that pyrophosphates are not entirely eliminated by fusion, especially if the fusion time is under two minutes. On standing, these samples become more intense in color, and in some cases after 24 hours the values are still low as compared to the results obtained with a longer fusion time or with acid hydrolysis of the sample. The effect of varying the time of fusion is shown by the data in table 1 secured on a sample of powder to which was added 5 p.p.m. of iron.

When the time of fusion was only one half minute, the percentage transmittance was decreased (a higher p.p.m. value was obtained) as the time interval elapsing between fusing and reading the spectrophotometer was increased up to 24 hours. When the fusion time was one minute, the percentage transmittance was greater after 4 hours than after 10 minutes, but there was no further significant change after 24 hours. When the fusion

time was 2–4 minutes, practically the same values were obtained whether the readings were made 10 minutes or 24 hours after fusing.

The dry ashing procedure, using hydrochloric acid to dissolve the ash and to eliminate the interference of pyrophosphates, was found to give satisfactory results. The ashed sample is hydrolyzed in the dish that is used for ashing so that there are no transfers necessary except to the volumetric flask. The method using acid hydrolysis is faster than the sodium carbonate fusion method. When using the former method, at least twelve to fifteen determinations can be made in a 4-hour period after the ash is removed from

TABLE 1

The effect of fusing time on the per cent transmittance of a sample of powdered milk over a 24-hour period

Sample	Fusion time	Transmittance 10 minutes	Transmittance 4 hours	Transmittance 24 hours
	min.	*per cent*	*per cent*	*per cent*
5210	½	91.0	81.0	73.3
5210	1	82.0	73.5	72.8
5210	2	71.0	70.5	70.8
5210	4	71.5	71.0	71.0
5210	Acid hydrolysis	70.5	70.5*	.....

* Read at one hour.

the muffle. The samples can be read within 10–15 minutes after the reagents have been added.

1,10-PHENANTHROLINE USED FOR COLOR DEVELOPMENT

For developing the color of the iron, it was decided to use 1,10-phenanthroline. Sandell (10) states that, for the most accurate determination of minute quantities of iron, the use of 1,10-phenanthroline is recommended. Fortune and Mellon (6), in a study of 1,10-phenanthroline using fifty-five possible interfering ions, found that there were very few ions that seriously interfered with the production of the quantitative color reaction. Of the few ions that were found to interfere, the only ones present in milk ash in significant quantities are pyrophosphates, copper, zinc and nickel.

The pyrophosphate interference can be eliminated by heating for 5 minutes with hydrochloric acid (1 + 1). Copper can be eliminated by controlling the pH between 2.5 and 4.0. The interference of zinc can be controlled by adding a larger excess of reagent. In this study it was found that nickel gave no interference when added in quantities up to three parts per million.

The advantages of the use of 1,10-phenanthroline in the determination of iron may be summarized as follows: (a) it gives a relatively more intense color for a given iron concentration (it gives approximately 9 per cent more

color as compared to 2,2′-bipyridyl); (b) it has almost complete freedom from interference by most of the common ions; (c) it has greater working sensitivity; (d) a color is produced that is more desirable for colorimetric comparison; (e) the orange-red complex produced is stable, and no fading has been observed for as long as six months; (f) pH need not be regulated closely; (g) color formations occur in acid solution, eliminating the difficulties usually caused by precipitation of metal hydroxides and hydrated oxides in alkaline solution.

The thiocyanate method according to Sandell (10) is still extensively used for the determination of iron, even though other reagents give better results. He also states that the reaction is a delicate one from the quantitative standpoint, because the color intensity depends upon a number of factors such as the excess of thiocyanate, the kind of acid present and the time of standing. The thiocyanate method is favored when the color reaction must be carried out at a low pH.

HYDROXYLAMINE HYDROCHLORIDE USED AS A REDUCING AGENT

Saywell and Cunningham (11), in their work on fruit juices, found a 10 per cent solution of hydroxylamine hydrochloride to be satisfactory as the reducing agent in the iron determination. Fortune and Mellon (6) tried using hydroxylamine hydrochloride, sodium sulfite, sodium and potassium formates, and formaldehyde as the reducing agents, but found the hydroxylamine hydrochloride solution to be the most satisfactory. Mehlig and Hulett (8) tried to use stannous chloride as the reducing agent, but also found it not to be as satisfactory as hydroxylamine hydrochloride. In the 2,2′-bipyridyl method used by the Quartermaster Corps (10), hydroquinone is used as the reducing agent. Hydroquinone could also be used as the reducing agent with 1,10-phenanthroline but, even though kept cool and in the dark, it will deteriorate in a few weeks.

Further advantages of the use of hydroxylamine hydrochloride are: (a) it does not have to be stored in the dark; (b) it is not necessary to keep it refrigerated; (c) because of its stability, a large quantity can be prepared at one time; (d) it rapidly reduces ferric to ferrous iron; (e) the color intensity of the complex formed between 1,10-phenanthroline and ferrous iron is independent of the acidity in the pH range 2–9. Below pH 2 the color does not develop, develops slowly, or does not fully develop (6, 11).

CONTROL OF pH

To eliminate the interference of copper the pH must be controlled between 2.5 and 4.0 (6). It is preferable to have the pH of the sample solution the same as that of the standards. The method was standardized so that the final pH of the solution and standards were 3.4 to 3.8.

OUTLINE OF RECOMMENDED PROCEDURE

I. *Apparatus Required:*

Platinum dishes (5 cm. diameter and 25–50 ml. capacity).

Platinum or glass-tipped tongs.

Photoelectric colorimeter (a Coleman Universal Spectrophotometer was used throughout this work).

Muffle furnace equipped with a rheostat and pyrometer.

Pyrex glassware.

II. *Reagents and Solutions:*

1. Acetate buffer solution. Reagent grade containing a maximum of 0.0005% iron. The buffer is made up by adding 16.6 grams of sodium acetate and 24 ml. of redistilled C.P. glacial acetic acid and diluting to 200 ml. with redistilled water in a volumetric Pyrex flask.

2. Hydroxylamine hydrochloride. C.P. 10% solution in water (10 grams hydroxylamine hydrochloride plus 90 grams of redistilled water).

3. 1,10-phenanthroline monohydrate. Saturated solution in redistilled water (1 gram 1,10-phenanthroline dissolved in 300 grams of water and warmed to effect solution). According to Smith (13) 1,10-phenanthroline is soluble to the extent of 0.016 Moles per liter at 25° C.

4. Water redistilled from Pyrex.

5. Concentrated ammonium hydroxide C.P.

6. Hydrochloric acid C.P. (1 + 1 solution by volume).

7. Redistilled nitric acid for cleaning glassware (redistilled from Pyrex).

III. *Preparation of a Standard Iron Solution:*

The standard iron solution is prepared by dissolving 0.1000 gram analytical grade iron wire in 20 per cent hydrochloric acid and carefully evaporating to dryness on a steam bath. The dried material is dissolved in a minimum amount of hydrochloric acid, transferred quantitatively to a 1,000-ml. Pyrex volumetric flask and diluted to volume. This stock solution will contain 100 micrograms of iron per milliliter. A stronger stock solution can also be prepared by dissolving 1.0 gram analytical grade iron wire and diluting to 1,000 ml. This stock solution will contain 1 milligram of iron per milliter.

To produce a working standard from the weaker stock solution, a 100 ml. aliquot is diluted to 1 liter. This gives a standard of 100 micrograms per ml. in solution. For the stronger solution mentioned above, a 10 microgram per ml. solution can be prepared by diluting 10 ml. of the stock solution to 1 liter.

IV. *Spectral Transmittance Curves:*

Spectral transmittancy curves are made for 20-microgram and 30-microgram iron solutions by adding the required amount of standard solution

and making it up to 10 ml. The iron solutions are made in iron-free Pyrex test tubes. The following are added—in the order indicated—to the 10-ml. iron solution, which is shaken after each addition:

2 ml. hydroxylamine hydrochloride
5 ml. acetate buffer solution
2 ml. 1,10-phenanthroline solution

The transmittancy curves for 20 micrograms and 30 micrograms of iron using a 2-cm. cell are shown in figure 1. The peak of the absorption band is located at 500 millimicrons. Twenty micrograms of iron give a transmittancy of 43.2 per cent while thirty micrograms of iron give a 29.0 per cent transmittancy. In these determinations a reagent blank using 10 ml. of redistilled water plus reagents was set at 100 per cent transmission.

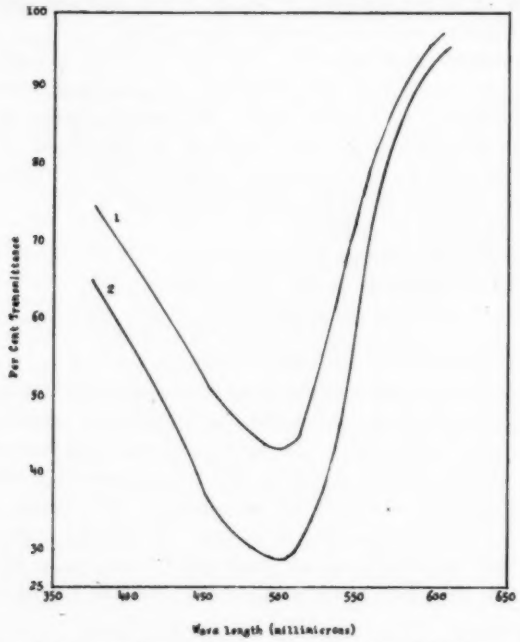

FIG. 1. Spectral transmittance curve for iron using 1,10-phenanthroline and a 2.0-cm. side cuvette. 1. 20 micrograms of iron. 2. 30 micrograms of iron.

V. Preparation of Standard Reference Curve:

Aliquots of 0.5, 1.0, 2.0, 3.0 and 4.0 ml. of the standard iron solutions (1 ml. = 10 micrograms) are accurately measured into iron-free Pyrex test tubes and diluted to 10 ml. with redistilled water, and the reagents are added as described in the spectral transmittancy curve. A blank, using 10 ml. of redistilled water, and the reagents are set at 100 per cent trans-

TABLE 2

Preparation of standard curve

Ml. standard solution (1 ml. = 10 micrograms)	Fe in 10 ml. solution	Transmittance
	p.p.m.	*per cent*
0.5	5	82.8
1.0	10	65.6
2.0	20	43.4
3.0	30	29.0
4.0	40	20.0

mittancy in the preparation of the standard curve. The data obtained for the standard curve using a 2-cm. cell and set at 500 millimicrons are given in table 2. These data were plotted on semi-logarithmic paper plotting per cent transmission against the concentration. The results are given in figure 2. It will be noted that the five points determined for this curve follow a straight line and thus conform closely to Beer's law.

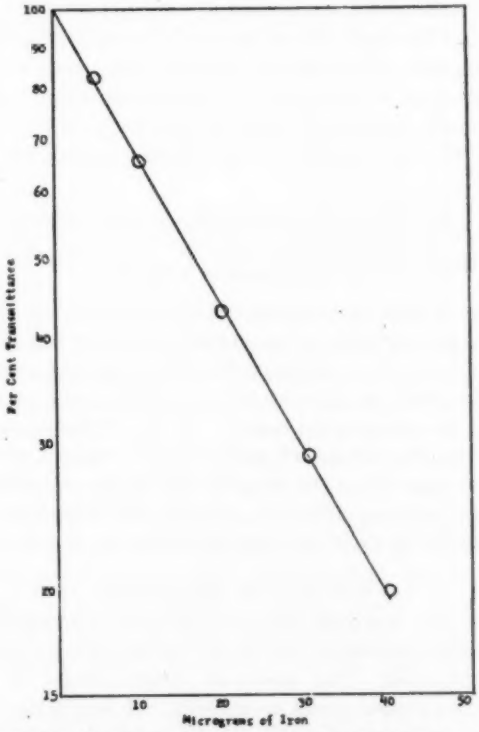

FIG. 2. Standard reference curve for iron using 1,10-phenanthroline, 2.0-cm. side couvette, and wave length set at 500 millimicrons.

VI. *Procedure for Iron Determination:*

Weigh out accurately 5 grams of powdered milk into a platinum dish or crucible. Place the dish or crucible into a muffle furnace having a temperature of not more than 300° C. When charring is complete (usually not more than an hour), gradually raise the temperature to 500–550° C. and ash until the sample is a greyish white color. With the type of platinum dishes used, it was found that 4 hours' ashing time after charring was sufficient. Or, if convenient, the sample could be ashed over night (16–18 hours). The ashed samples are removed from the muffle and allowed to cool.

The dish is placed on a clay triangle, and 5 ml. of hydrochloric acid (1 + 1) is added. The sample is then covered with a watch glass and gently boiled for 5 minutes. When the dish and watch glass have cooled, the watch glass is removed, and any droplets formed on it are washed into the dish with redistilled water.

The solution is then transferred, by means of a pipette, a Pyrex funnel or other convenient means, to a 50-ml. volumetric flask. Redistilled water is placed in the dish, which is then heated. The water is added to the 50-ml. volumetric flask. The flask is filled almost to the neck with redistilled water, 1 ml. of concentrated ammonium hydroxide is added and the contents mixed. The flask is then filled to the mark with redistilled water. A 10 ml. aliquot of the prepared ash solution is pipetted into an iron-free Pyrex test tube and the following color reagents are added, the solution being shaken after each addition:

2 ml. hydroxylamine hydrochloride solution
5 ml. acetate buffer solution
2 ml. 1,10-phenanthroline solution

A permanent orange-red complex $[(C_{12}H_8N_2)_3Fe]^{++}$ develops if any iron is present, the intensity being proportional to the iron content.

The iron content of the platinum dish and reagents are determined the same way as the ashed sample and used as a blank and set at 100 per cent transmission in the spectrophotometer. In this determination of iron, the spectrophotometer was set at 500 millimicrons (using a PC–4 filter), and a 2-cm. cell was used as in the preparation of the spectral and standard curves. Since a one-gram aliquot is used in this determination, the p.p.m. iron is read off directly from the standard reference curve.

DISCUSSION OF THE METHOD

In this study, flat platinum ashing dishes with a diameter of 5 cm. were used. Vycor dishes were tried on a number of samples but consistent results could not be obtained. The platinum dishes should be handled with platinum- or glass-tipped tongs to prevent contamination. To clean the platinum dishes, hydrochloric acid (1 + 1) should be boiled in them until they are clean and are iron-free.

It is imperative that all water used be as free from iron as possible. Water redistilled from Pyrex is practically iron-free.

Pyrex glassware should be used throughout the determination. All reagents should be prepared and stored in Pyrex, and need not be refrigerated. The glassware should be cleaned by rinsing with distilled nitric acid, followed by a number of times with distilled water and finally redistilled water.

It was found that a satisfactory blank could be obtained by using reagent grade sodium acetate. A comparison of purified with reagent grade chemicals showed that the amounts of iron present were practically identical.

By ashing under a temperature of 550° C. and hydrolyzing for five minutes with hydrochloric acid (1+1), no interference from pyrophosphates was encountered. If it is desired to make sure that no pyrophosphates are present, the sample should be read again after one hour.

After the addition of the one milliliter of concentrated ammonium

TABLE 3

The recovery of added iron in the analysis of powdered milk

Sample No.	Fe added	Cu added	Fe calculated	Fe found	Recovery
	p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*	*per cent*
1022	0	0	.....	6.7	.....
1022	5	1	11.7	11.6	99.14
1022	10	2	16.7	16.4	98.20
1022	15	3	21.7	21.4	98.61

hydroxide to the aliquot in the 50-ml. flask, a white precipitate may form which may be redissolved by the addition of a few drops of hydrochloric acid (1+1).

Loss of iron on ashing and interference from copper. A factor reputed to cause analytical errors is the loss of iron by volatilization during the ashing process. Andrews and Felt (1) have reviewed some of the investigations that deal with this problem, and there seems to be no agreement whether or not there is a loss of iron by volatilization. Andrews and Felt, in the recovery of added iron in the analysis of flour and bread, show that there is no evidence of any loss of iron since the difference between the found and calculated values is within range of the probable error.

The writers have investigated the loss of iron as evidenced by the recovery of iron added to the sample before ashing. The iron solution added was that prepared for the standard reference curve. Copper (copper wire standard) was also added to these samples to note whether there was any interference from copper. The results for the iron recoveries are given in table 3.

When 5 to 15 parts per million of iron and 1 to 3 parts per million of copper were added to powdered milk before ashing, recoveries from 99.14 per cent to 98.20 per cent were obtained. Similar excellent recoveries have

TABLE 4

Effect of length of ashing time on iron content of powdered milk

Sample No.	Ashing time	Iron (sample not filtered)	Iron (sample filtered)	Fe
	hrs.	*p.p.m.*	*p.p.m.*	*p.p.m.*
3	3	4.9	4.4	.....
3 + {1 p.p.m. Cu, 5 p.p.m. Fe}	3	10.4	9.4	.....
3	4	.....	.....	4.4
3	18	.....	.....	4.5
3 + 5 p.p.m. Fe	18	.....	.....	9.6
3 + {1 p.p.m. Cu, 5 p.p.m. Fe}	18	.....	.....	9.6

been obtained on other samples of milk powder with iron added before ashing. There seems to be no interference from copper in the 1,10-phenanthroline method at the pH used.

Effect of length of ashing time. There was evidence throughout the investigation that, in the preparation of the sample for analysis, it is important to get the sample completely ashed. Incompletely ashed samples usually produced high results as the carbon interfered with the color produced. With the flat type of platinum dish used in this study, it was found that four hours' ashing (after charring is complete) is sufficient to produce a clear sample. Some typical results obtained on 4- and 18-hour ashing and on an incompletely ashed sample are given in table 4.

Effect of length of hydrolysis time on the recovery of iron in powdered milk. In the early study of this method it was found that inconsistent results would be obtained if the hydrolysis time was limited to a few minutes. To note the effect of hydrolysis time, a sample of powdered milk was hydrolyzed from 5 to 20 minutes after ashing for 4 hours (from time of complete charring). The results are given in table 5.

Five minutes' hydrolysis time was found to be sufficient to produce the quantitative color reaction within 10 minutes after the reagents are added. The only objection to longer hydrolysis time is the mechanical losses that may occur. There was no interference from pyrophosphates, as the color did not change after the samples were read again in one hour.

TABLE 5

Effect of length of hydrolysis time on the iron content of a sample of powdered milk

Sample No.	Hydrolysis time	Iron, 10 minutes	Iron, 1 hour
	min.	*p.p.m.*	*p.p.m.*
4	5	5.0	5.0
4	10	5.1	5.0
4	15	5.0	4.9
4	20	5.0	5.1

TABLE 6

The effect of the order of adding the reagents on the rate and intensity of the color development

Sample No.	Order of adding reagents	Iron, 10 minutes	Iron, 1 hour
		p.p.m.	*p.p.m.*
5210	RA–B–OP*	4.4	4.4
5210	B–RA–OP	4.4	4.6
5210	RA–OP–B	4.4	4.6
J12	RA–B–OP	6.9	6.9
J12	B–RA–OP	6.7	6.7
J12	RA–OP–B	6.7	6.7

* RA = Hydroxylamine hydrochloric reducing agent; B = Sodium acetate buffer; OP = 1,10-phenanthroline.

Effect of order of adding reagents on iron content of powdered milk. Banderer and Schaible (3) made a critical study of the 1,10-phenanthroline method and state that sodium citrate is more satisfactory in adjusting the pH than sodium acetate, and that the order of adding the reagents influences the rate of color development. They used hydroquinone as a reducing agent. Sodium citrate cannot be used when hydroxylamine hydrochloride is used as the reducing agent (4). To note the effect on the rate of color development, the sodium acetate buffer was added in various orders to the sample. The results on two samples of powdered milk are given in table 6.

With the use of hydroxylamine hydrochloride as the reducing agent, it does not seem to matter in what order the sodium acetate is added, as the maximum color is produced within 10 minutes. However, since a standard procedure is desirable, it is recommended that the reagents be added in the following order: (1) reducing agent; (2) sodium acetate buffer; and (3) 1,10-phenanthroline, as this seemed the most logical order of adding the reagents.

Variations in the iron content of a commercial batch of powdered milk. It was noted that, in analyzing samples prepared with the pilot spray drier at the University of Illinois, invariably the first batch of powder made was higher in iron than succeeding batches. These results were checked at a commercial plant where 66 barrels of whole milk powder were made from one batch of milk. Samples were taken out of the first barrel and a number of other barrels throughout the run. Results are given in table 7.

TABLE 7

The iron content of a run of powdered milk

Barrel	Iron found
	p.p.m.
1st	4.6
23rd	3.8
44th	3.6
66th	3.3

The first milk through the system produced a powder higher in iron than samples taken from the barrels later in the run. There was a gradual decrease in the iron of this powder as the run continued. This is an important point to keep in mind when samples are taken by regulatory agencies.

SUMMARY

A rapid, accurate method for the determination of iron using hydroxylamine hydrochloride, sodium acetate buffer, and 1,10-phenanthroline is described. The ash is taken up with hydrochloric acid (1+1) and heated for 5 minutes. No interference from pyrophosphates, copper and nickel was observed. In the amounts found in milk, zinc did not interfere with the color development. An excess of 1,10-phenanthroline is added to take care of any zinc complex that may be present.

The reagents used in this iron determination are stable and need not be refrigerated or stored in the dark. The advantages of the use of 1,10-phenanthroline and hydroxylamine hydrochloride are given.

Losses of iron during ashing have not been observed.

The order of addition of the sodium acetate buffer was not found to affect the intensity of the color produced.

The variations of the iron content of a batch of commercial powdered milk are given.

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ANNOUNCEMENT

AMERICAN DAIRY SCIENCE ASSOCIATION

1945 MEETING CANCELLED

The War Committee on Conventions has denied the American Dairy Science Association a permit to hold the 1945 meeting scheduled for Iowa State College, Ames, Iowa, June 12–14. The meeting has therefore been cancelled by unanimous vote of the Board of Directors. We all realize the importance of the Dairy Industry in the present war emergency and the value of the contributions made to the advancement of Dairy Science through personal, group and open discussion in a national meeting. However, all of us will be glad to cancel this meeting to assist even in the slightest way in aiding the prosecution of the war.

Your Board of Directors, the Program Committee and Iowa State College have made every effort to have things in readiness so that if it proved possible to hold a meeting they would not have been negligent in any particular. In the face of difficulties we all recognize in the present situation, a splendid war-time program had been arranged through the fine cooperation of our membership. It had been agreed that all addresses, abstracts and reports would be published in the August issue of the Journal of Dairy Science as usual even if the meeting was cancelled. It has been suggested, however, that the authors be given an opportunity to decide whether they wish to submit abstracts and this is being done.

The Board plans to meet in Chicago, June 12 and 13, to take care of all necessary business matters. The splendid cooperation and support of our membership in a year when there have been conflicting views and many difficulties to overcome is greatly appreciated.

The 1946 meeting will be held at Iowa State College, Ames, Iowa, in June 1946, on dates to be announced, war conditions permitting.

A. C. RAGSDALE, *President*

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The Royal Technical College, Copenhagen, Denmark

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ABSTRACTS OF LITERATURE

BOOK REVIEW

92. Chemistry and Technology of Food and Food Products, Vol. II. Published by Interscience Publishers, Inc., 215 Fourth Avenue, New York City. MORRIS B. JACOBS, EDITOR. 892 pages, including index, 1944. $10.50. (Set of two volumes, $19.00.)

This is the second of two volumes which contain four of the six parts of a review of food technology practices. These are: unit operations and processes applicable to typical food products, maintenance of sanitary and quality control of foods and food products, the principal methods of preserving foods, and production methods for principal foods. This volume is divided into twenty-five chapters written by twenty-seven collaborators representing the knowledge and experiences of research, production and legal aspects of food technology. These chapters cover the physical processes and procedures in treatments given foods, food supervision by government agencies, food grading, types and application of food machines, cleaning methods and principles, insect and rodent control, food preservation by: dehydration, temperature control, in hermetically sealed containers, by use of microorganisms, chemical preservatives, and packaging; typical production and processing methods are illustrated in chapters on cereal grains and its products, including breakfast cereals, flours, bread and bakery products; sugars and syrups; confectionery and cocoa products; fruit juices, jams, jellies, preserves; principles and related products of meat, oils, and fats, milk, alcoholic and nonalcoholic beverages, and industrial waters. A number of the chapters are well illustrated by diagrams or photos; the bibliography of each chapter is limited to a brief but selected list. The chapters are subdivided with convenient subtitle headings.

Even though there have been several new texts in various phases of food technology published recently, this work by a number of collaborators has enabled much new material and new viewpoints to be presented. For those in the dairy industry the volumes definitely will be of value for guidance in the development of new products, and in the problems and methods of use of milk in food products. K.G.W.

BACTERIOLOGY

93. A Sterile Cutting Device for Swab Vial Outfits Utilizing Wood Applicators. THEODORE C. BUCK, JR., AND EMANUEL KAPLAN, Assistant Director and Chief, Division of Chemistry, Bureau of Laboratories, Baltimore City Health Dept., Baltimore, Md. Jour. Milk Technol., *7*, No. 3: 141. May–June, 1944.

A description and illustration of the cutting device is given. L.H.B.

BREEDING

94. A Survey of Results of Artificial Breeding of Dairy Cattle in New Jersey. E. J. Perry and J. W. Bartlett. New Jersey Agr. Expt. Sta. Cir. 489. 8 pages. Nov., 1944.

A summary of replies by 112 association members to a 24-question survey made to learn the chief results of artificial breeding in New Jersey since its beginning in 1938. In general the members expressed satisfaction with results; in the light of the knowledge gained six problems are listed as important for study in connection with artificial breeding associations. Topping this list is the problem of finding outstanding bulls that are highly fertile. J.G.A.

BUTTER

95. Only Butter Can Be Butter. Owen M. Richards, Amer. Dairy Assoc., Chicago, Ill. Natl. Butter and Cheese Jour., *36*, No. 3: 24. Mar., 1945.

Americans prefer butter to oleo and the American Dairy Association is attempting to foster this preference by advertising, by urging the increase of the butter supply through better returns for butter producers, and by showing that butter is the "balance wheel" of the dairy industry.

W.V.P.

96. Problems in Churning Diverted Cream. J. C. Marquardt, N. Y. State Dept. of Agr. and Markets, Albany 1, New York. Natl. Butter and Cheese Jour., *36*, No. 2: 22. Feb., 1945.

During June and July, moisture, fat, curd and salt analyses were made on 61 samples of the butter made from diverted fluid milk. The results were reported to the creameries with suggestions for improvement. Subsequently 40 samples of butter were analyzed from the same factories. The statistics show desirable trends in composition control following the suggestions. Fat losses in buttermilk were 1% to 2% in some churnings but separator losses of fat were not excessive. W.V.P.

97. Butter vs. Vegetable Spreads in the Post-War Period. H. A. Ruehe, American Butter Institute, Chicago, Ill. Natl. Butter and Cheese Jour., *35*, No. 11: 26. Nov., 1944.

Milk is a natural food never duplicated by man, and the fat of milk is distinguished from other fats because it contains short-chain fatty acids and is naturally highly palatable. Vegetable spreads have their place in the market but should be sold for what they are. Those which have been so

marketed may become more popular as new flavors and fruits become available. Others which imitate butter in characteristics as well as use should be controlled to insure citizens against fraud. W.V.P.

98. Starters and Cultures. M. W. HALES, Chr. Hansen's Laboratory, Inc., Milwaukee, Wis. Natl. Butter and Cheese Jour., *35*, No. 7: 26, July, 1944, Part III; No. 8: 24, Aug., 1944, Part IV; No. 9: 44, Sept., 1944, Part V; No. 10: 45, Oct., 1944, Part VI; No. 11: 78, Nov., 1944, Part VIII.

Part III. Preferred equipment for carrying mother cultures includes pressure cookers or autoclave, although a steam cabinet will do, Pyrex flasks stoppered with cotton or paper caps, nine-gram pipettes for transferring inoculations and a culture cabinet. Bulk starter requires an insulated, stainless-steel starter-can or well-tinned milk cans and an insulated steam and water-tight tank to hold the cans. It is ideal to have a separate starter room. The milk for cultures and bulk starter may be mixed milk from several herds but more careful selection and higher quality may be found in milk from one or two patrons. Since many properties of milk influence starters, it is best to select milk of high solids content from healthy well-fed cows not too early or late in lactation.

Part IV. Activity of cultures may be lost by bacteriophage contamination with dust or other phage-laden material. Phage also causes irregular, unpredictable, culture activity. The phage so easily contaminates cultures that a separate starter room is desirable.

It is best to select milk for starters from mixed milk of several good herds and to sterilize it.

Part V. Sterilizing of milk for mother cultures is recommended but heating enough to form a dark brown color tends to inhibit growth of starter organisms. Low-temperature heat treatments produce weak-bodied starter; best body follows heating to 185° F. for 30 minutes; heating to 225° F. for 15 minutes gives weak body and caramelized color and flavor. Size of inoculation should be adjusted to give a 12- to 16-hour incubation period at 70° F., and for this purpose 0.5 to 1% is suggested to give proper growth of both acid and flavor-producing organisms.

Part VI. Incubation of cultures and starters should be at 70° F. for proper flavor, body, activity, elimination of thermodurics, and judging of quality. Degree of ripening may vary with the purpose of using starter; low-acidity starters contain less volatile acids and are generally favored by cheesemakers.

Part VII. Over-ripening may cause flavor loss by increasing acidity and decreasing acetylmethylcarbinol and diacetyl. Some operators cool cultures as soon as they coagulate to maintain activity, others develop 0.8% acid; variations depend on characteristics desired. Chilling when acidities are

over 0.7% is recommended. Cultures should be kept at 40° F. or lower until used. A recapitulation of 15 important points to observe in starter-making concludes this series. W.V.P.

CHEESE

99. The Cheese Industry of the Future. RAYMOND MIOLLIS, Natural Cheese Co., Shelbina, Mo. Natl. Butter and Cheese Jour., *36,* No. 3: 40. Mar., 1945.

In 1950 the house wife will insist on pasteurized-milk cheese just as she now insists on pasteurized milk. Pasteurization will eliminate under-grade cheese of all types, will simplify packaging of natural cheese, and will encourage the making of new varieties. Compulsory pasteurization in general will require larger plants, better equipment, and highly skilled technicians but small plants making high-priced specialties for a limited market can compete if properly managed. W.V.P.

100. Effect of Vacreation of Milk on Quality of Cheddar Cheese. R. P. ROBICHAUX, Cherry-Burrell Corp., Chicago, Ill. Natl. Butter and Cheese Jour., *35,* No. 11: 68. Nov., 1944.

This is a progress report of work completed when the author was a research assistant at Oregon Agr. Expt. Station. The work shows that strong feed flavors are removed without churning the fat but with softer curd formation, less matting tendency on cooking, better closing in the press and improvement in quality. The heat treatments were, for preheating, 145° F. and, for vacreating, 165° F. for not more than 2 seconds. Vacreated milk required more rennet for proper coagulation. W.V.P.

101. Can Pasteurized-Milk Cheese Compete with Raw-Milk Cheese? WALTER V. PRICE, Univ. of Wis., Madison, Wis. Natl. Butter and Cheese Jour., *35,* No. 11: 32. Nov., 1944.

Pasteurized-milk cheese can compete: for milk, because pasteurized milk can be used more efficiently to make a more uniform product of high quality; for labor, because it simplifies and systematizes daily operations; for a market, because of consumer preferences for mild cheese of uniform flavor; for the approval of health officials, because it insures destruction of harmful organisms in the milk at the time of delivery. These conclusions are not intended to detract from the merits of raw-milk cheese carefully made from an approved milk supply. W.V.P.

CHEMISTRY

102. Use of Lactobacillus Arabinosus 17-5 for Microassay of Pantothenic Acid. EDWARD H. HOAG, HERBERT P. SARETT, AND VERNON

H. CHELDELIN, Oregon State College, Corvallis, Ore. Jour. Indus. and Engin. Chem., Indus. Ed., *17*, No. 1: 60. Jan., 1945.

L. arabinosus used as the test organism in a microbiological assay method for pantothenic acid showed greater and more rapid response in the range 0.01 to 0.08 micrograms of pantothenic acid than did *L. casei*. Assay values with good recoveries of added pantothenic acid were obtained turbidimetrically after 14 hrs. growth, or titrimetrically after 24 to 30 hrs. The pantothenic acid values of foodstuffs as determined by *L. arabinosus* and *L. Casei* showed good agreement. Assay values of yeast concentrates by the chick method were higher than those obtained microbiologically. B.H.W.

103. Gel-Forming Derivative of Wheat Gluten. HENRY C. REITZ, ROBERT E. FERREL, AND HAROLD S. OLCOTT, Western Regional Research Laboratory, U. S. Dept. of Agr., Albany, Calif. Jour. Indus. and Engin. Chem., Indus. Ed., *36*, No. 12: 1149. Dec., 1944.

When wheat gluten is reacted with chlorosulfonic acid in pyridine or with cold concentrated sulfuric acid, a gel-forming product can be recovered. Upon addition of cold water, this material absorbs almost instantly from 100 to 300 times its weight of water to form a firm, odorless, tasteless, and nontoxic gel. The use of gluten sulfate as a substitute for scarce natural gums in therapeutic jellies, ointments, and other pharmaceutical preparations and as a thickening or emulsifying agent in foods is suggested. Since the gels are not affected by freezing its use as an emulsifier or thickener in ice cream is mentioned. B.H.W.

DISEASE

104. Mastitis in Dairy Cattle. T. M. OLSON and F. M. SKELTON. S. Dak. Agr. Expt. Sta. Cir. 54. 12 pages. Aug., 1944.

A popular treatise which summarizes the salient facts on mastitis as follows:

Mastitis is usually caused by a specific organism, *Streptococcus agalactiae*. It occurs in one or more of the milking cows in nearly all herds of any size. It is more prevalent in old than in young cows. The most common symptoms of mastitis are: swollen, inflamed quarters; flakes or lumps of curd in the milk and on the milk strainer pad. Mastitis-producing bacteria enter the udder through the streak canal. There are several tests for detecting mastitis in milking cows. The microscopic inspection of milk in which the long-chain streptococci organisms are found is the most dependable. Mastitis can be controlled by testing, observing sanitary practices in the barn, use of medicaments, and slaughter of the chronic cases that will not yield to treatment. J.G.A.

105. Bloat in Dairy Cattle. T. M. Olson. S. Dak. Agr. Expt. Sta. Cir. 52. 11 pages. June, 1944.

A popular presentation of the problem setting forth four different theories as to the cause, and suggesting preventive measures in lieu of a definitely established and dependable remedy which as yet has not been found. J.G.A.

106. Gramicidin and Tyrothricin Therapy in Chronic Streptococcic Mastitis. Ralph B. Little, Dept. of Animal and Plant Pathology, Rockfeller Institute for Medical Research, Princeton, N. J. Milk Plant Monthly, *33*, No. 10: 31–34. Oct., 1944.

Mastitis in a herd represents an economic loss through reduction in milk yield, and, through elimination of udder-diseased cows from the herd each year. At least 15% of the annual replacements are necessary because of mastitis. Much progress has been made in chemotherapeutic treatment of chronic mastitis during the past three years. Good results have been obtained by injecting directly into the udder cistern small amounts of bactericidal agents without infusing the entire quarter. Such procedure offers the advantages of time-saving, less equipment required, and simplification in sterilization when a great number of cows are to be treated. Gramicidin is an alcohol-soluble, water-insoluble substance extracted from cultures of a soil bacillus, *Bacillus brevis*. The crude material extracted from the bacillus is called tyrothricin from which two different crystalline products, tyrocidin and gramicidin, are obtained. Both of these products have been rather extensively used by workers in the United States in the treatment of streptococcic mastitis. Some workers have reported an efficacy of over 90% in the sterilization of infected quarters.

The author makes twelve recommendations which are most pertinent to the question of udder therapy. These should be followed closely if the most satisfactory results are to be obtained. The use of bactericidal agents now available for udder medication cannot be considered as a cure-all, but simply as an adjunct to be used in the eradication of the disease. Emphasis is placed on the importance of the dairyman adopting proper control and sanitary measures, which are so essential in the identification and isolation in the care of normal and infected members of the herd before udder medication is begun. Seventeen references are given. G.M.T.

107. A Study of the Effectiveness of Sulfonamide Preparations in the Elimination of Bovine Mastitis. W. W. Swett, R. R. Graves, C. A. Matthews, J. F. Cone, and P. C. Underwood. U. S. Dept. of Agr. Tech. Bul. 884. 20 pages. Dec., 1944.

Progress report on the first ten months of a study in which 176 cows were

used. The preparations studied were sulfonilamide-in-oil, and sulfadiazine-in-oil.

Because mastitis interferes with milk secretion its control is of great importance, especially in wartime when there is need for increased quantities of dairy products to meet the demand. The high degree of effectiveness of the sulfonamide preparations used (especially those containing sulfadiazine) in treating staphylococcal infections, the rarity of unfavorable reactions, and the lack of any significant depressing effect on milk secretion resulting from administration of the treatments indicate the high merits of these preparations for use in eliminating mastitis infections—particularly streptococcal, staphylococcal, and coliform infections. J.G.A.

FEEDS AND FEEDING

108. Defluorinated Superphosphate for Livestock. R. B. Becker, G. K. Davis, W. G. Kirk, R. S. Glasscock, P. T. D. Arnold and J. E. Pace. Florida Agr. Expt. Sta. Bul. 401. 15 pages. Aug., 1944.

This study was made because of the acute wartime shortage of bonemeal for livestock feeding. Fifty-six beef steers, twenty-four dairy heifers, and ten pigs were used as subjects. The authors note that although defluorinated phosphate is not palatable when fed alone, it is being used successfully in mixed feeds. Detailed findings were as follows:

Defluorinated superphosphate being produced commercially contains 10.9 to 14.0 per cent of phosphorus, 25 to 28 per cent of calcium, and less than 1.2 per cent of fluorine. This mineral should substitute safely for steamed bonemeal in feeding livestock. Under range conditions cattle consumed satisfactory amounts of Supplement B, containing 45 parts of defluorinated superphosphate, although they showed some preference for Supplement A, containing steamed bonemeal. Beef cattle on improved pastures refused to take plain defluorinated superphosphate. They preferred mixtures with a large proportion of salt over those containing less salt. Dairy heifers on improved pastures in central Florida chose a 7:3 mixture of salt and defluorinated superphosphate over 4 others with progressively lesser proportions of salt, while having free access to common salt and No. 1 "salt sick" mineral. Dairy cows on improved pastures adjacent to brackish water expressed a decided preference for a 3:7 mixture of salt and defluorinated superphosphate and for the No. 2 "salt sick" mineral, over 4 other mixtures containing progressively more salt. They had access to steamed bonemeal, common salt and No. 1 "salt sick" mineral at the same time. Pigs grazing peanuts ate slightly more of swine mineral containing bonemeal than another group of pigs consumed of swine mineral with defluorinated superphosphate. They made slightly better gains also, but the differences were insufficient to be significant, based on 1 trial. Sows and boars found the experimental

swine mineral to be palatable. Defluorinated superphosphate can be used satisfactorily with swine, beef and dairy cattle when offered in appropriate form. Its selection should be governed by convenience and economic reasons. J.G.A.

109. Comparison of Molasses-Soybean Silage and Corn Meal-Soybean Silage as Feeds for the Milking Cow. W. A. KING. New Jersey Agr. Expt. Sta. Bul. 713. 20 pages. Nov., 1944.

Twenty cows (12 Holsteins and 8 Guernseys) were used in this study for a period of 15 weeks. Rates of silage preservatives used were: corn meal, 250 lbs. per ton of green soybeans and molasses, 80 lbs. per ton.

The addition of corn meal to soybeans resulted in a well-preserved and very palatable silage. Molasses-soybean silage was also well preserved, but was not so palatable as the corn meal silage or as corn silage. The soybean silages preserved by corn meal and by molasses were found to be as valuable as corn silage in the rations of milking cows. There was no significant difference in the economy of milk production of the cows fed these silages. For the average cow producing 25 or more pounds of milk daily on roughage alone, soybean silage preserved with 250 pounds of corn meal per ton of green material fed with hay was found to be impractical. The apparent digestibility and the apparent metabolizable energy of the corn meal-soybean silage were higher than those of the molasses-soybean silage, but not so high as had been expected. Chemical analyses, results of the feeding trial, and the total digestible nutrient values of the corn meal silage indicate a loss of nutrients of approximately 10 per cent in excess of normal silage losses. The molasses-soybean silage was less digestible and contained less metabolizable energy when fed to milking cows as the sole feed than was similar silage fed as a part of a complete ration. The coefficient of digestibility of the crude fiber of the corn meal silage was significantly lower than that of the molasses silage. The importance of nitrogen analyses on fresh samples of silage and feces is emphasized. If these losses had not been recognized there would have been a significant error in the coefficient of digestibility of the crude protein and in the nitrogen balances of the cows. Nitrogen balance studies indicate that the protein of the soybean silage was inadequate as the main source of protein in rations of milking cows. The calcium and phosphorus of the soybean silages were well utilized by the cows. J.G.A.

FOOD VALUE OF DAIRY PRODUCTS

110. Milk, Nutrition and Human Health. Dr . J. M. SHERMAN, Chief, Dept. of Dairy Industry, Cornell University, Ithaca, N. Y. Milk Dealer, *33*, No. 11: 96–100. Aug., 1944.

"The health of our people, sound economics, and common sense all call

for more milk, not less, during the present period." The post-war outlook in occupied Europe will be the nutritional need for more milk and milk products to supply the "not so hidden hunger" needs of a semi-starved peoples. While the American people are better fed than those of Europe, malnutrition due to inadequate nutritional diets is still our greatest medical problem. To solve this problem would be a vast economic gain for the country as a whole. It can be most economically solved by increasing milk in the diet to at least a pint a day for each American. An improvement in our dietary habits by use of milk has been quantitatively proven. Calcium deficiencies, as one of the most important "hidden hungers" is most readily solved by increased milk intake. Riboflavin deficiencies are likewise corrected most expeditiously by increased use of milk and dairy products. Milk and milk products also economically supply Vitamin A. The importance of quality in dairy products must be recognized. Good milk, as well as more milk, is essential, for people cannot be forced to use more milk—they must want more milk. Only quality will create such a desire. The greatest need both from the standpoint of the welfare of the American people and from the point of view of the dairy industry is therefore to put it bluntly, "to get more milk in the human stomach." This result can best be obtained through basic research and through education of the mass of the people. Bread is the staff of life but through "refinements" in milling, the staff became "badly bent if not quite broken." To incorporate more milk in bread would put more milk in the human stomach and thereby make the staff of life more nutritious and hence enhance the nutritional balance needed in American life. C.S.T.

111. **Effect of Feeding Dried Milk on Production of Liver Cancer by p-Dimethylaminoazobenzene.** KANEMATSU SUGIURA, Memorial Hospital, N.Y.C. Soc. Expt. Biol. and Med. Proc., *57*, No. 2: 231. Nov., 1944.

Experiments were conducted to determine the effect of dried milk feeding on the incidence of hepatic tumors in rats after feeding p-dimethylaminoazobenzene. The production of liver cancer was partially inhibited by the daily ingestion of dried whole milk (50 per cent protection at 200 days). Liver cirrhosis was treated successfully by a rice diet containing 15 per cent dried whole milk. Once adenomatous hyperplasia of bile ducts, cholangioma, or hepatoma was established in the livers they could not be destroyed by ingestion of the rice-milk diet. R.P.R.

112. **Significance of the Protein Level in Synthetic Diets.** THEODORE F. ZUCKER AND LOIS ZUCKER, Columbia University. Soc. Expt. Biol. and Med. Proc., *55*, No. 2: 136. Feb., 1944.

Evidence was presented to show that liver factor, which permits optimal

growth in male rats fed a diet containing 18 per cent purified casein and 6 pure B factors, may not easily be found in the usual concentrated sources of B complex. It was found, however, that the factor is associated, either as an impurity or an essential amino acid, with protein of good quality.

R.P.R.

ICE CREAM

113. **Home Freezers.** DONALD K. TRESSLER, Manager General Electric Consumers Institute, Bridgeport, Connecticut. Ice Cream Trade Jour., *41*, No. 1: 32. Jan., 1945.

The early home freezers built prior to 1937 were inefficient due to the difficulty of obtaining an effective moisture vapor seal around the outside, of the insulation. By 1938 freezers were constructed by some manufacturers of refrigeration equipment in sizes varying from 2 to 50 cubic feet capacity. Since the war started, very few home freezers have been built. Some ice cream cabinets have been used but these are ill adapted for the purpose because they were designed to maintain a temperature of approximately 10° F., a temperature somewhat too high for the rapid freezing and storage of foods.

One of the hazards in home freezing is that of overloading, resulting in spoilage before the food is brought down to 32° F. It is not necessary to quick freeze the foods, but they should be chilled to a temperature of below 50° F., prior to being placed in the cabinet and the amount of food put in the cabinet at any one time limited to an amount which will be reduced in temperature from 70° F., to 10° F., in not more than 24 hours when the cabinet is operating in a 100° F., room.

Freezing may be accelerated by placing a small fan in the freezer or by placing the food to be frozen on a metal plate in which a refrigerant is being circulated.

A temperature of approximately 0° F., should be maintained in the freezer in order that the flavor, color and vitamin content of the food may be preserved. Standards for home freezers have been suggested by the Rural Electrical Administration.

There are some hazards in home freezing of foods, due to mechanical and power failures. However, food will not thaw to any considerable extent until 72 hours after the current is off. In general, it is a bad practice for commercial freezers of food to attempt to refreeze them after they thaw due to shrinkage and poor appearance of the food and package. W.H.M.

114. **Continuous Freezers.** E. H. FORSTER, Cherry-Burrell Corp.,Chicago, Ill., Ice Cream Trade Jour., *41*, No. 1: 26. Jan., 1945.

The degree of efficiency of a continuous ice cream freezer depends on the

composition, freezing and whipping characteristics of the mix and on a clean efficient refrigeration system of ample capacity.

The presence of excessive oil in the ammonia system, improperly sharpened or dull blades and insufficient refrigeration in the plant or inefficient operations of the refrigeration equipment are important factors in continuous freezer operations and control.

The usual reason for excessive oil in the ammonia system are: 1. High discharge temperature on compressor. 2. Too high an oil level in crank case of splash system compressors, and ammonia in crank case. 3. Worn or broken piston rings and cylinders that allow the compressor to pump oil. 4. Absence of oil traps, oil traps of inadequate size or failure to drain oil traps.

A continuous freezer blade is considered to be sharp when the heel or wearing surface is approximately 1/64″ wide. The easiest way to keep continuous freezer blades in proper condition is to touch them up with a fine mill file or oil stone after every two or three days of operation, or after every 15 to 20 hours of operation. When sherbets and ices are frozen in long runs the blades will need more frequent attention. Detailed instruction with illustrations of proper methods of sharpening blades can be obtained from the freezer manufacturer.

Efficient operation of all refrigeration equipment is also required for uniform continuous freezer operation. The following conditions affect uniform operation: 1. Insufficient ammonia in the refrigeration system or excessive flash gas in ammonia fed to freezer. 2. Sluggish float valve staying closed until most of the ammonia in the freezer is used up and then opening and admitting a large amount of warm liquid ammonia.

Many continuous freezer operation and control problems are the result of underfreezing or conditions which cause difficulty in getting the ice cream stiff enough to prevent thorough and uniform air incorporation. In addition to low heat transfer rate in the freezer, there are other factors which result in underfreezing. Introduction of the mix into the freezer at too high a temperature, low fat and low solids and mixes of low viscosity may also result in under freezing. The use of superheated condensed skim milk or ceral products in the mix may greatly improve freezer operation.

W.H.M.

115. Looking Ahead in Packages. VINCENT M. RABUFFO, Editor, Ice Cream Trade Jour., *40*, No. 12: 26. Dec., 1944.

Adequate low-temperature storage facilities which will become available after the war in the new household refrigerators with their low-temperature compartments and the new home freezers and storage cabinets, should make it possible for ice cream manufacturers to greatly expand the volume of ice cream sold for home consumption.

Refrigerator manufacturers estimate that during the five-year period after the war they may produce 15,000,000 new household units, each, with ¾ to 1½ cubic feet of low-temperature space. Predictions vary on the production of home freezers but estimates are for a production of about 2,000,000 units during the first 2 years after the war. Because of the anticipated development in home refrigeration, food stores and large super-markets are contemplating the sale of packaged ice cream. This should have a beneficial effect on all other methods of selling and dispensing ice cream because it will create new customers and existing customers will be educated to greater consumption. Price and quality were mentioned as factors which would influence consumer acceptance of the post-war packaged ice cream. Lower over-run than that which has prevailed in the past was recommended (not more than 65 per cent). At present the manufacturers get a higher price for packaged ice cream; however, it may be possible in the future to reduce costs, by effecting economies in the entire packaging operation. As at present the pint and quart package will probably continue to be most popular with the consumer. W.H.M.

116. **Ice Cream's Opportunity.** A. J. CLAXTON, Vice President and General Sales Manager, Beatrice Creamery Co., Chicago, Ill. Ice Cream Trade Jour., *40*, No. 12: 28. Dec., 1944.

There are many problems in the distribution of ice cream which face the ice cream industry, in the future. The industry has enjoyed a 100 per cent increase in consumption since 1920, as a result of the war it has been necessary to limit ice cream production and this has resulted in an unsatisfied demand on the part of the American people for good ice cream. When peace comes the ice cream manufacturer will have a magnificent opportunity to satisfy this demand.

During the war the ice cream manufacturer has been faced with increased costs due to the use of costlier dairy products, improved machinery, high wages and increased taxes. These increases have been met partly by the sale of reduced-product-cost items and by economical delivery. In the future production costs are likely to continue to increase but by careful planning it may be possible for the industry to retain some worth while gains in distribution costs. Recommendation included the manufacture of fewer and better items, elimination of special deliveries, and other wasteful practices. W.H.M.

117. **Selling in the Future.** ELMER J. MATHER, Vice Pres., National Dairy Products Corp. Ice Cream Trade Jour., *41*, No. 1: 18. Jan., 1945.

Post-war sales policies should be fair to consumers, fair to dealers and fair to the ice cream manufacturer. This means the sale of good product and adequate service at a reasonable price. As soon as restrictions are lifted

the ice cream industry should lose no time in restoring quality and making even a better product than was made in pre-war days. Good raw materials, proper formula and good equipment operated by properly trained and supervised employees are all essential in producing quality ice cream. After a manufacturer has established his wholesale price he should inform the public of the retail price and encourage his dealers to sell his particular brand at the same retail price. Ice cream prices are also affected by the manufacturer's cabinet and service policy. Special service, unnecessary deliveries, oversize cabinets and other concessions to the dealer must be reflected in the selling price. Such practices should be discouraged because they cost the consumer money. More important than these in gaining consumer acceptance for ice cream, is to get the dealer to pay attention to store sanitation, personal hygiene of employees and proper methods of dispensing. Ice cream manufacturers in the past have been called on to make loans to dealers for the purchase of equipment. This practice is to be discouraged since it can be handled better through the banks, the manufacturer will have more capital available for the purchase of new trucks, and equipment which will be needed in the post-war period.

Ice cream manufacturers should also give some consideration to the training of salesmen. Such a program should include information about the product, how it should be handled by the dealer so that he can make a profit. In the post-war period it will be necessary to protect the dealer's business and assist him to get new business in his neighborhood. W.H.M.

118. Auditing Ice Cream. R. A. LARSON, Ohio State University, Columbus, Ohio. Ice Cream Trade Jour., *41*, No. 1: 22. Jan., 1945.

Since 1936 Ohio State University has conducted what they called an ice cream audit. Interested manufacturers send pint samples of ice cream or sherbet to the Department of Dairy Technology. The samples are tested for weight, fat, total solids, bacteria, *E. coli*, and scored for flavor, body and texture and color. A charge of one dollar per sample is made for the analysis. The results of the analysis and scoring of all samples are summarized and sent to all who submitted samples. This gives each manufacturer an opportunity to check his ice cream against all others submitted and has done much to improve and standardize Ohio ice cream. W.H.M.

MILK

119. Fieldman's Share in Producing Quality Milk—XIII. Is Aeration of Milk Necessary? G. M. TROUT, Mich. Agr. Expt. Sta. Milk Plant Monthly, *33*, No. 10: 22–25. Oct., 1944.

The author has made an extensive review of the literature, citing forty-one references on the subject. It was pointed out that aeration of milk was

considered more important years ago than at the present time. At best aeration removes only part of the off-odors of milk. Rather than aerating milk to remove odors, the odor should be avoided through proper milk production. During the past score of years marked improvements have been made on the quality of milk production on the farm. This has resulted in large part because of (a) investigations concerning the causes and prevention of off-flavors in milk due primarily to feeds; (b) construction of better-ventilated dairy stables; (c) changed practices of feeding in respect to time of milking; (d) development of milk-cooling units; (e) universal tendency toward prompt handling and cooling of milk; (f) introduction of new cleaning and sterilizing compounds; and (g) closer inspection of milk supplies. The investigations, developments and introductions have contributed much toward the production of milk remarkably free from odors which were once thought to be an inherent part of milk production and which could be removed only by aeration. G.M.T.

120. Fieldman's Share in Producing Quality Milk—XIV. Problems of the Mechanical Milker. LARRY E. PEW, Hinman Milker Representative. Milk Plant Monthly, *33*, No. 11: 26–27. Nov., 1944.

Despite forty years of development, the mechanical milker still has its problems. Chief of these is the fact that to be successful over a period of time it must work in cooperation with the cow. It is the only labor-saving machine on the farm which operates on living flesh. Until recently engineers who developed the mechanics of the present-day machines had little real knowledge of the dairy cow upon whose cooperation the success of the machine depended. In servicing the machines the author finds that the faults of the milker fall into three classes, volume, vacuum and faulty units. However, the all-important factor in the success of a milker is the operator himself. Too many operators expect the milker to do the entire job itself without any particular care on their part. There is a permanent place for the mechanical milker in the dairy industry, but the machines must be built to operate with the cow and not from a mechanical standpoint alone. Therefore, the operator remains an important unit in the use of the machine.
G.M.T.

121. Fieldman's Share in Producing Quality Milk—XV. Dairy Farm Supplies, Their Distribution and Use. M. P. BAKER, Iowa State College. Milk Plant Monthly, *33*, No. 12: 26–27. Dec., 1944.

Farm dairy supplies, such as washing powders, brushes, chlorine, filter pads, may well be handled by the distributor, in order to furnish the producer with the best products for the production of high quality milk with the least cost and labor possible. Furnishing these materials to the pro-

ducer alone is not enough. The fieldman should instruct the producer as to the best methods of using the equipment and keeping it in first-class condition. Washing and sterilizing utensils are separate operations. Sterilizing may be accomplished with hot water or with chlorine. Hot water should be above 170° F. and should be in contact with surface of the utensils for at least two minutes. Rinsing is not as effective as immersing. Sterilization of dairy utensils in the strictest word is not complete. Therefore, utensils should be stored to dry to prevent bacterial growth. Chlorine solution should be sufficiently strong that at least 50 parts per million of available chlorine is present after sterilization. Chlorine sterilization should be done immediately prior to the use of the utensil. The chief object of the distributor handling the supplies is to secure the production of good quality milk economically. G.M.T.

122. **Does Your Can Washer Wash Cans Clean?** ANONYMOUS. Natl. Butter and Cheese Jour., *35*, No. 11: 30. Nov., 1944.

The can washer cannot function properly if it is not properly serviced. Such service requires proper water softening, thorough rinsing after each use, scrubbing off of scale and sludge, and proper lubrication. W.V.P.

123. **The Paper Milk Bottle.** T. H. BROUGHTON, Manager Detroit Milk Bottle Exchange. Milk Plant Monthly, *33*, No. 12: 25–32. Dec., 1944.

Paper containers are used very extensively in Los Angeles, Detroit, Chicago, New York, and Washington. Little, or practically no paper containers, are used in Pittsburgh, Philadelphia, Toledo, and St. Louis. The first major installation of paper equipment in Detroit was in 1937. By 1943 ten Detroit dealers put out approximately one hundred million units in paper. Paper containers for milk seem to fit best in markets having large wholesale business, or in certain types of transportation. Important factors to consider in judging the value of paper milk containers are as follows: paper weighs less, saves space and from the customer's standpoint has the advantage of not having to be returned. The advantages of glass are, first, economy, and, second, visibility. Some believe that use of paper saves labor, and that the matter of economy was not so important. During the war years with the shortage of labor the trend in the sale of milk has been toward the wholesale business which is a type most favorable to paper. Results of studies indicate that the cost per unit for paper containers is approximately 4/10 of a cent more than the comparative cost for glass bottles. This figure applies only to large operations, the smaller dealer having a much higher unit cost. Some smaller Detroit dealers using preformed paper containers must necessarily pay as much as two cents per unit, thus representing a container cost 1½ cents more than similar costs using glass. G.M.T.

124. Indirect Advertising of Milk and Milk Products. L. M. DORSEY, Prof. of Dairy Husbandry, Univ. of Maine, Orono, Me. Milk Dealer *34*, No. 1: 44–45, 70–74. Oct., 1944.

Despite the fact that not one principal dairy product is advertised in a leading household magazine, a survey of its advertising columns reveals that 25 proprietary products, wherein only 8 contain milk products, mention milk or dairy products as part of their advertising. These advertisements range alphabetically from the American Meat Institute to Wheaties and Wesson Oil, and from nursing bottles to Frigidaire refrigerators where milk and milk products are cited in their utilization—all gratis advertising yet none of the several national organizations representing the major branches of the dairy industry do any direct advertising of the products they represent. The nutritional aspect of milk and milk products could well be advertised and told in household magazines with an eye to the future. The author points out that even the advertisement of oleomargarine, an out and out substitute for butter, stresses the fact that skim milk is used to impart "a delicate natural flavor" and that dog food contains cheese to impart a cheese flavor. The implication of the article is that milk and milk products as such carry some of the advertising load and not to depend solely on proprietary products or other national associations non-dairy in scope to provide all the extolling of the nutritional or culinary values of milk and milk products, or in other words, start supplying the wind to "toot our own horn" even though under existing OPA, ODT, and WFA restrictions and regulations, it is impossible to fill all consumers' orders at the present time.

C.S.T.

125. Modern Milking Methods. R. M. CARTER, University of Vermont Agr. Ext. Service Circular 111. 14 pages. Nov., 1944.

Contains detailed instructions for care and operation of the milking machine, preparation of the cow, stripping, and other aspects of quick milking.

J.G.A.

126. Labor Usage in Milking. S. W. WILLIAMS. Vermont Agr. Expt. Sta. Bul. 517. 14 pages. Oct., 1944.

This study of milking machine operation on 79 farms shows that the length of time the machines are on the cows and the amount of stripping done vary widely from farm to farm, and that averages for both are higher than good milking practice would indicate. Among the herds vistited, the machine time averaged 6.5 minutes per cow; the stripping time, 1.6 minutes; and the weight of strippings, 1.2 pounds. The length of time the machines were left on apparently did not affect the duration of stripping or the weight of the strippings. Furthermore, neither the make or age of the machine nor

the average milk production per cow was appreciably related to either the speed or the completeness of the machine milking. Men who operated three or more single units, or their equivalent in double units, and did their own stripping and caring for milk, left the machines on longer, but stripped less, than those handling two or fewer units per man. There was wide variation among farms both in time spent and in distance walked in performing each of the separate jobs involved in milking. Among 14 herds milked by machine, total milking time ranged from 3.0 to 8.1 minutes, and walking from 78 to 209 feet, per cow. There was similar variation among hand-milked herds. These differences in labor requirements seem due in the main to differences in the practices which individuals regard as necessary parts of their milking procedure, in building arrangement, in equipment, and in the care with which the work is planned. On the basis of experience on one Vermont farm, where changes in method, barn arrangement, and equipment were made and their effect on time and travel observed, it appears that proper attention to these points makes possible important savings of labor.

J.G.A.

127. Costs of Producing Milk in Pennsylvania. W. L. Barr. Pennsylvania Agr. Expt. Sta. Bul. 467. 36 pages. Aug., 1944.

A dairy farm arrangement survey was made on 348 farms in the summer of 1942 in order to determine the costs of milk production. The various items of cost were grouped under three major headings; feed, man labor, and other costs. Total feed and bedding accounted for 51.3 per cent of the costs; man labor, 32.8 per cent; and other costs, 15.9 per cent. The net cost of producing a hundredweight of milk totaled $2.94. Marketing costs of 23 cents added to the net cost of production made the total cost of producing and marketing milk $3.17 per hundredweight. On the average, 2.8 hours of man labor, 35.5 pounds of concentrates, 54.5 pounds of hay, 15 pounds of other roughage, and 92.6 pounds of succulents were used to produce a hundredweight of milk. According to the methods of calculating costs used in this study, there was a net loss of 52 cents per hundredweight of milk produced. When all expenses other than for man labor were deducted from total returns, the net amount remaining to pay for labor averaged 20 cents per hour. Thus the average return to labor was actually only about one-half the 37 cents it was calculated to have cost.

Returns per hour of labor which were less than the calculated cost of man labor meant that on the average farmers did not receive sufficient income to offset the calculated costs of all items. Home-grown feeds were charged at prices reported as being paid for those items at the farm. Labor was charged at a calculated rate which was a combination of cash expense and what the farmers estimated unpaid labor and perquisites were worth. Producers did not receive the values at which the above and other items were charged to the dairy.

J.G.A.

128. Cost of Processing Chocolate Milk. GIDEON HADARY. Milk Dealer *34*, No. 1: 47–48. Oct., 1944.

Three hundred eighty-four million quarts of chocolate milk, a tenfold increase in ten years, were processed in the United States in 1942. This increase was largely due to the relative profitability of this product as compared with other milk products handled by the milk dealer. Eight milk dealers in Wisconsin ranging in volume production from less than 250,000 pounds to over 500,000 pounds per month volume were surveyed for cost data. A table showing difference between cost of handling a quart of chocolate and plain milk is given which shows additional costs per quart of chocolate milk ranged from 1.746 to 3.019 cent per quart with an average of 2.581 cents. Additional handling costs for chocolate milk comes from (1) the loss from returns and (2) the equipment and general overhead chargeable to the product. Returned chocolate milk may be re-pasteurized and re-sold so that returns can be used. The differential cost, if any, would therefore be less than 0.09 cent. The additional cost of processing chocolate milk usually would be offset by (1) higher price charged for chocolate milk (1 to 3 cent per quart) and (2) lower butterfat content of chocolate milk. The butterfat content ranged from 1.5 to 3.5% at milk plants studied. The difference in cost per quart as compared with plain milk ranged from 0.71 to 2.31 cents per quart. The main additional costs involved in processing chocolate milk are: (1) flavoring and (2) inside labor costs. C.S.T.

129. Producing Milk with Low Bacterial Counts. M. J. PRUCHA AND J. M. BRANNON, Dept. of Dairy Husbandry, University of Illinois, Urbana, Ill. Jour. Milk Technol., *7*, No. 3: 129. May–June, 1944.

This report shows what can be accomplished in a small plant in a small community by a dairyman, who was greatly interested in distributing milk to his customers containing less than 1,000 bacteria per ml. L.H.B.

130. Reliability of Formulae in Determining Milk Solids-Not-Fat. FRED A. WIGGERS, Laboratory Director, Toledo Health Dept., Toledo, Ohio. Jour. Milk Technol., *7*, No. 3: 135. May–June, 1944.

Comparison of three formulae (Babcock #1, Babcock #2, and Hawley-Davis) were made with a standard method.

Nine samples of pasteurized whole milk were examined to accurately determine the percentage of solids-not-fat by using as a standard for comparison total solids as determined by dehydration in an air oven (according to Standard Methods) minus the butterfat as obtained by the Babcock test.

Both of the Babcock formulae gave consistently lower results than the standard method.

The Hawley-Davis formula checked closest with the standard method; however, it was recommended that whenever an accurate determination of the percentage of solids-not-fat was desired. it was best to use the standard procedure rather than a formula. L.H.B.

131. **Supplementary Home Sanitation of Shipping Cans.** M. C. JAMIESON AND H. K. CHEN, Dept of Animal Pathology and Bacteriology, The University of Manitoba, Winnipeg, Canada. Jour. Milk Technol., *7*, No. 3: 136. May–June, 1944.

Four sanitizing agents were used to treat 364 cans. All of the agents studied were almost equally effective in reducing the bacteria to practical insignificance in commercially washed cans.

Swab tests were made on the cans 15 hours after washing. The cans were then sprayed (wetting all surfaces) with the sanitizing agents (4 oz. to a can). After 15 minutes the cans were dried over a warm air jet and reswabbed. Care was taken not to reswab the same part of the can that was swabbed for the first test. L.H.B.

132. **Conservation of Milk Plant Equipment.** C. W. WEBER, State Dept. of Health, Albany, N. Y. Jour. Milk Technol., *7*, No. 3: 156. May–June, 1944.

The life of equipment is affected by physical factors, such as friction and shock and chemical action or corrosion. It is advisable to have a lubrication chart for each piece of equipment showing where and when to grease, the kind and amount of grease to use and when the service was last performed.

Pipes, pipe fittings and valves should be handled with care. Use rubber mallet to loosen can tops.

Keep equipment painted to prevent corrosion. Use proper washing compounds of proper strength. Cans and equipment should be dried after washing.

Train new help thoroughly. L.H.B.

133. **The Small Town Milk Control Unit.** C. A. HOOVEN, Marshalltown, Iowa. Jour. Milk Technol., *7*, No. 3: 160. May–June, 1944.

An explanation is given of how six communities having a total population of slightly over 41,000 have pooled their resources, which enabled them to employ an experienced milk sanitarian and establish laboratory control of their milk supply.

In these six communities there are sixteen pasteurizing plants obtaining milk from one hundred farms, and fourteen raw milk dealers distributing milk from sixteen farms.

In the past year about 1500 agar plates and about the same number of microscopic examinations were made. Routine phosphatase tests have been

made on all pasteurized samples and butterfat tests were made on all bottled samples of milk collected.

The U. S. Public Health Service Ordinance is in effect, and the cost has been about the price of a quart of milk per person per year. L.H.B.

134. **Selling the Milk Sanitation Program to the Public.** LEWIS BLEVINS, Dept. of Public Health, Pratt, Kansas. Jour. Milk Technol., *7*, No. 3: 162. May–June, 1944.

Four points are discussed that are important in selling the program. 1. The sanitarian must sell himself to the industry. 2. The governing body of the community must be sold on the importance of the program. 3. Sell the newspapers. 4. Sell the public. L.H.B.

PHYSIOLOGY

135. **Relation of Certain Endocrine Glands to Body Weight in Growing and Mature Guinea Pigs.** J. P. MIXNER, A. J. BERGMAN, AND C. W. TURNER, Univ. of Mo. Endocrinology, *32*, No. 3: 298. Mar., 1943.

Pituitary, thyroid, adrenal, and gonad weights of 207 male and 146 female growing guinea pigs were related to their body weights by using the relative growth equation $Y = aX^b$. The equation was fitted to the data by the method of least squares. Equations and graphs for predicting the various endocrine gland weights from body weights were presented. R.P.R.

136. **Determination of the Rate of Thyroid Hormone Secretion at Various Environmental Temperatures.** E. W. DEMPSEY AND E. B. ASTWOOD, Harvard Medical School. Endocrinology, *32*, No. 6: 509. June, 1943.

Young male rats, weighing 80 to 120 gm. at the beginning of the experiment, were used. The maintenance of restoration of normal thyroid weight by the administration of thyroxin to rats simultaneously fed the antithyroid drug, thiouracil, was used as the basis of an assay technique for thyroid hormone. The rate of thyroid enlargement in response to thiouracil feeding was low in hot environments and high in the cold. The quantity of thyroxin required to maintain a thyroid of normal weight at various temperatures was: 5.2 micrograms at 25° C.; 9.5 micrograms at 1° C.; and, 1.7 micrograms at 35° C. These values were considered to be quantitatively equivalent to the amount of hormone produced by the normal thyroid gland under these conditions. Metabolic rate determinations gave a figure in substantial agreement with that determined by the prevention of thiouracil-induced hyperplasia. R.P.R.

137. Nature of the Pituitary Factor Stimulating Mammary Duct Growth. J. J. TRENTIN, A. A. LEWIS, A. J. BERGMAN, AND C. W. TURNER, Univ. of Mo. Endocrinology, *33*, No. 2: 67. Aug., 1943.

Normal male mice were used for the assay of various pituitary preparations for their mammary duct growth factor. The mice were injected subcutaneously on 6 successive days and killed on the seventh day. The formation of end buds on any one or more of the mammary rudiments was taken as a criterion for a positive response. Using improved extraction procedures, the bulk of the activity of the fresh pituitary tissue was recovered in the protein fraction rather than in the lipid fraction. R.P.R.

138. Effects of Adrenal Cortical Compounds on Lactation. WARREN O. NELSON, ROBERT GAUNT, AND MALVINA SCHWEIZER, Wayne University Medical School and New York University. Endocrinology, *33*, No. 5: 325. Nov., 1943.

Young guinea pigs, of either sex and weighing from 300 to 450 gm., were prepared for lactation by daily injection with estrogen. Two or 3 days thereafter hypophysectomy was performed and various replacement therapy instituted. Lactation was not induced by the lactogenic hormone alone but was initiated if either the adrenotrophic hormone or adrenal cortical extract was injected with the lactogen. Lactation was not induced when desoxycorticosterone acetate was given with adequate amounts of lactogen. Lactation was induced when 17-hydroxy-11-dehydrocorticosterone was adminstered with lactogen. It was suggested in hypophysectomized animals and demonstrated in intact animals that 3 mg. daily of desoxycorticosterone acetate has an inhibiting influence on lactation. It was thought that this effect was related to the fact that mammary growth could be induced by this steroid when administered in daily doses of 2 mg. R.P.R.

139. Stimulation of Mammary Glands in Hypophysectomized Rats by Estrogen and Testosterone. S. L. LEONARD, Cornell Univ. Endocrinology, *32*, No. 3: 229. Mar., 1943.

Estradiol dipropionate stimulated growth of the end buds of mammary glands of hypophysectomized immature male and female rats if: (1) rats weighed less than 70 gm. at the time of hypophysectomy; (2) the injections were begun immediately after the operation; and (3) the glands were examined after 10 to 12 days of treatment. Mammary gland stimulation was not obtained in hypophysectomized rats if: (1) the rats were heavier than 70 grams at the time of the operation regardless of when hormone treatment was begun; and (2) a period of 7 days elapsed before treatment was begun regardless of age or weight of rat. It was suggested that mammary gland cells retain, for a certain period of postnatal life, potentialities for growth

which can be stimulated by estrogen, but after this critical period, these potentialities are lost forever and the cells are unaffected by estrogen. Injections of testosterone propionate into young hypophysectomized male rats resulted uniformly in thickening of the mammary gland ducts. This thickening was caused by some hyperplasia and by hypertrophy of the epithelial cells, and by an increase in the diameter of the lumina of the ducts. Secretory material was observed in many ducts of the stimulated glands and was thought to form from the cytoplasm of the epithelial cells.

R.P.R.

140. Environmental Modification of Certain Endocrine Phenomena. EDWARD W. DEMPSEY AND HAROLD F. SEARLES, Harvard Medical School. Endocrinology, *32*, No. 2: 119. Feb., 1943.

Experiments were reported which demonstrated that endocrine activity may be modified by exteroceptive stimuli. Transection of the pituitary stalk in a group of female rats resulted in unaltered, normal cycles in approximately half of the animals. Pseudopregnancy, production of deciduomata, and short periods of vaginal cornification were produced in rats with sectioned pituitary stalk after estrogen injections. Maintenance of female rats in constant light caused constant follicular development without ovulation; however, ovulation and pseudopregnancy were induced by sterile mating. High environmental temperatures caused a decrease of the thyroid-cell height but exposure to cold resulted in increased thryoid-cell height and enlarged adrenals. Simultaneous exposure to cold and light induced constant estrus as well as adrenal and thyroid stimulation. With the exception of the thyroid stimulation by exposure to cold, all of these reactions were produced in rats after pituitary stalk section. It was concluded that environmental stimuli may be transmitted to the anterior pituitary by routes other than the pituitary stalk and that various endocrine components react in unitary fashions when the endocrine balance is altered.

R.P.R.

141. Non-Specific Results Obtained with Micro-Method for Assay of Prolactin. ERNEST L. LAHR, ROBERT W. BATES, AND OSCAR RIDDLE, Carnegie Institution of Washington. Endocrinology, *32*, No. 3: 251. Mar., 1943.

Plucking feathers incident to making local cropsac tests for the lactogenic hormone led to a slight, temporary increase in the rate of cell division in the underlying epithelium of the crop. A further slight and temporary increase in mitotic rate in the glandular epithelium was produced by splitting the skin or by intradermal injections of 0.5 cc. of control substances such as air, water, and 0.9% NaCl. Many substances tested at 48 hours after their first injection were apparent irritants since they produced a positive macroscopic response together with an increased mitotic count.

Doses of 1 to 2 mg. of the wound substance, leukotaxine, produced a prompt increase in the rate of mitosis observed at 2 to 24 hours but not 48 hours thereafter. R.P.R.

142. Relation of Certain Endocrine Glands to Body Weight in Growing and Mature New Zealand White Rabbits. H. H. KIBLER, A. J. BERGMAN, AND C. W. TURNER, University of Missouri. Endocrinology, *33*, No. 4: 250. Oct., 1943.

Rabbits used in the study were fed a ration of mixed grain and alfalfa hay, kept at an environmental temperature between 50 and 70° F. and killed during the fall, winter, or early spring. Body weights were recorded to the nearest gram and the thyroid, paired adrenals, and gonads were weighed to the nearest 0.1 mg. The relative growth equation $Y = aX^b$ was used to relate the thyroid, adrenal, and gonad weights of 320 male and 362 female growing and mature rabbits to their body weights. The equation was fitted to the data by the method of least squares. The exponents b of 0.88 of the thyroid and 0.94 for the adrenals of animals weighing less than 2500 gm. indicated that the ratio of the weights of these glands to body weight decreased as body weight increased. For animals weighing over 2500 gm. the exponents b of 1.78 for the adrenals, 2.04 for the ovaries, and 2.84 for the testes indicated that the ratio of the weights of these glands to body weight increased as body weight increased. R.P.R.

143. The Effect of Various Gonadotropic Hormone Preparations on the Blood Plasma Ascorbic Acid of Sheep and Rabbits. HENRY A. LARDY, L. E. CASIDA, AND PAUL H. PHILLIPS, University of Wisconsin. Endocrinology, *35*, No. 5: 363. Nov., 1944.

The subcutaneous administration of 400 I.U. of pregnant mare serum proved effective in reducing the blood plasma ascorbic acid in ewes. The average decrease was 23 per cent and the concentration remained essentially unchanged for 16 days. Repeating the dose brought about a transient rise in the blood plasma ascorbic acid level. A similar dose of pregnant mare serum decreased the blood plasma ascorbic acid of both pseudopregnant and anestrous rabbits. A follicle-stimulating extract made from sheep pituitary glands decreased the blood plasma ascorbic acid of ewes when injected on either the 4th, 8th, or 12th day of the estrous cycle. An unfractionated pituitary extract decreased blood plasma ascorbic acid of ewes and the normal level of blood plasma ascorbic level of ewes appeared to be higher on the 12th than on the 4th or 8th day of the estrous cycle. R.P.R.

144. Nitrogen Balances on Rats During Pregnancy and Lactation. L. M. MORSE AND C. L. A. SCHMIDT, University of California Medical School. Soc. Expt. Biol. and Med. Proc., *56*, No. 1: 57. May, 1944.

Nitrogen balances were carried out on rats maintained on four different

diets (synthetic diet, synthetic diet plus methionine, synthetic diet plus liver extract, and stock diet) during the period of pregnancy and lactation. The synthetic diet plus liver extract compared most favorably with the stock diet in preventing loss of nitrogen in pregnant and lactating rats.

R.P.R.

145. Effect of Massive Doses of Thiamine on Fertility and Lactation in the Albino Mouse. LEOPOLD R. CERECEDO AND LEONARD J. VINSON, Fordham University. Soc. Expt. Biol. and Med. Proc., *55*, No. 2: 139. Feb., 1944.

Albino mice were divided into 3 groups and fed the following rations: group 1, basal diet (finely ground Purina dog chow) plus 250 mg. thiamine per kilo of diet; group 2, basal diet plus 250 mg. thiamine plus 1 mg. $MnCl_2$ per kilo; group 3, basal diet plus 1 mg. $MnCl_2$ per kilo. The effects of the diets were studied throughout 3 generations. The massive doses of thiamine had no harmful action on reproduction and lactation. R.P.R.

146. Inhibitory Effect of Certain Amino Acids on Growth of Young Male Rats. STANLEY W. HIER, CLAIRE E. GRAHAM, AND DAVID KLEIN, The Wilson Laboratories, Chicago, Ill. Soc. Expt. Biol. and Med. Proc., *56*, No. 2: 187. June, 1944.

Gelatin did not permit optimum growth of young male rats even when supplemented with all reported essential amino acids. Less growth was obtained with gelatin plus amino acids than with the amino acids alone at the same level in the diet. Six per cent glycine, 5 per cent dl-phenylalanine, or 5 per cent l-proline in certain diets had an inhibitory effect on growth. It was thought that the inhibitory effect of glycine and proline on growth might explain the difficulty in supplementing gelatin for optimum growth.

R.P.R.

147. On the Function of the Ruptured Ovarian Follicle of the Domestic Fowl. IRVING ROTHCHILD AND R. M. FRAPS, U.S.D.A. Soc. Expt. Biol. and Med. Proc., *56*, No. 2: 79. June, 1944.

Evidence was presented to show that the ruptured follicle of the hen participates in the control of the time of lay of the egg normally arising from ovulation of its previously contained yolk. Removal of the ruptured ovarian follicle either alone or with the oldest maturing follicle at the time when the egg taking its origin from the ruptured follicle was in the oviduct invariably resulted in the holding of the egg beyond the expected time of lay. Most eggs were held from one to 7 days. Removal of other parts of the ovary practically never resulted in comparable holding of the oviducal egg. R.P.R.

148. The Mechanism of Action of Thiouracil. W. C. DIETRICH AND R. BEUTNER, The Hahnemann Medical College of Philadelphia. Soc. Expt. Biol. and Med. Proc., *57*, No. 1: 35. Oct., 1944.

Employing the Reid Hunt technique, a method whereby as little as 1 mg. of whole thyroid gland can be detected since it imparts to mice a distinct resistance against otherwise fatal doses of acetonitrile, it was shown that thiourea and thiouracil in addition to preventing the synthesis of the thyroid hormone interfere with the action of orally administered thyroid. R.P.R.

149. Effect of Dietary Protein on Liver Riboflavin Levels and on Inactivation of Estradiol by Liver. K. UNNA, H. O. SINGHER, C. J. KENSLER, H. C. TAYLOR, JR., AND C. P. RHOADS, Merck Institute for Therapeutic Research, Rahway, N. J., and the Memorial Hospital for Treatment of Cancer and Allied Diseases, New York City. Soc. Expt. Biol. and Med. Proc., *55*, No. 4: 254. Apr., 1944.

Weanling rats of both sexes maintained on a low protein diet were unable to retain normal amounts of riboflavin in the liver. Methionine tended to counteract the decrease in the liver riboflavin concentration. The livers of rats maintained on a low protein diet for 3 months failed to inactivate estradiol but supplementation with methionine maintained the ability to inactivate estradiol. R.P.R.

150. Mammary Development in the Thyroprived Bovine by Stilbestrol and Thyroprotein Administration. W. E. PETERSEN, C. B. KNODT, T. M. LUDWICK, AND B. S. POMEROY, University of Minnesota. Soc. Expt. Biol. and Med. Proc., *57*, No. 3: 332. Dec., 1944.

Two thyroidectomized cows with marked clinical myxedema were subjected to diethylstilbestrol administration alone and then in combination with thyroprotein. No mammary development was obtained by diethylstilbestrol or thyroprotein administration. Negative results were also obtained with the simultaneous administration of the two hormones as long as myxedema persisted. Substantial mammary development and lactation resulted from diethylstilbestrol treatment after the myxedematous symptoms had been relieved by thyroprotein therapy. It was suggested that the failure of mammary development in the myxedematous animals was due to some phase of the myxedema and that the response following thyroid therapy was not due to a synergistic action of thyroxine and diethylstilbestrol. R.P.R.

151. Alterations in Mammary Structure Following Adrenalectomy in the Immature Male Rat. CHARLES F. REEDER AND S. L. LEONARD,

Cornell University. Soc. Expt. Biol. and Med. Proc., *55*, No. 1: 61. Jan., 1944.

Bilateral adrenalectomy in either normal or castrated immature rats stimulated lateral bud growth of the mammary duct system and in some instances increased end bud growth, particularly when body growth was not markedly inhibited. Estrogen injections in adrenalectomized rats caused either dilatation of the lateral buds or further increased their number above that of the injected unoperated controls. R.P.R.

152. **Pituitary Stimulating Property of Stilbestrol as Compared with That of Estrone.** O. Watkins Smith and George Van S. Smith, Fearing Research Laboratory, Brookline, Mass. Soc. Expt. Biol. and Med. Proc., *57*, No. 2: 198. Nov., 1944.

Stilbestrol was found to be about 100 times as active as estrone in causing the stimulation and release of adrenotrophic and gonadotrophic factors from the pituitaries of intact mature male rats. In castrated rats it was only about twice as active as estrone. It was thought that the effect of stilbestrol upon the pituitary gland was direct rather than through the formation of some metabolic inactivation product. R.P.R.

153. **Differential Concentration of Hormones in the Central and Peripheral Zones of the Bovine Anterior Pituitary Gland.** George K. Smelser, Columbia University. Endocrinology, *34*, No. 1: 39. Jan., 1944.

The thyrotrophic, adrenotrophic, and gonadotrophic hormones were all found in greater concentration in extracts of tissues removed from the basophilic central zone than in the more peripheral and predominantly acidophilic and chromophobic cortical zone of the beef anterior pituitary gland. The ratio of basophilic material contained in any preparation of central and peripheral tissue was fixed, nevertheless the concentration of these hormones in center: cortex ratios was not the same (gonadotrophic 2:1; adrenotrophic 6:1 or 8:1; thyrotrophic 16:1 or 32:1). It was suggested that these hormones are produced or stored by several distinct cell types which have different spatial distributions in the gland. R.P.R.

154. **Effect of Graded Doses of Thyroxine on Metabolism and Thyroid Weight of Rats Treated with Thiouracil.** E. P. Reineke, John P. Mixner, and C. W. Turner, University of Missouri. Endocrinology, *36*, No. 1: 64. Jan., 1945.

An attempt was made to establish more fully the quantitative relation between the increase in metabolic rate and the decline in thyroid size of animals given thiouracil plus graded doses of thyroxine. Young male

albino rats were given 0.1 per cent thiouracil in their drinking water for a period of 2 weeks to block the formation of thyroxine. The effect of thiouracil alone and thiouracil plus graded doses of thyroxine on the metabolic rate and the thyroid gland weight was determined. Metabolic rate was depressed 23.7 per cent and thyroid weight was more than doubled in the rats receiving thiouracil alone. The daily injection of 4.75 micrograms of d, l-thyroxine returned the metabolic rate to normal. Approximately 4.8 micrograms of d, l-thyroxine was required to return the thyroid weight to normal. From their results the authors believe that thyroid assays or measurements of thyroid function determined by the new thiouracil technique are directly comparable with results obtained by the standard metabolic method. R.P.R.

155. Prolactin Assay by a Comparison of the Two Crop-Sacs of the Same Pigeon after Local Injection. S. R. HALL, U.S.D.A. Endocrinology, *34*, No. 1: 14. Jan., 1944.

Lactogen preparations were assayed by comparing responses in the two crop-sacs of the same young pigeon following local administration of the hormone. A 20 per cent change in dosage was detected in the 8 crop-sacs of 4 birds in both 2- and 4-day tests. A change of 100 per cent in dosage was not uniformly detected by assays in groups of pigeons. In the administration of lactogen solutions two factors were found to affect the response: (1) location on the skin of the injection site with respect to the underlying crop-sac; and, (2) the volume of the solution injected. Nearly equal responses occurred following both subcutaneous and intradermal injections. In the preparation of pituitary tissue two factors were found to affect response: (1) the use of suspensions of either acetonedried or fresh tissues instead of extracts; and, (2) acetone desiccation. The volume of extracting fluid was not a factor affecting the response. R.P.R.

156. Study of the Crop-Sac Weight Method for Prolactin Assay. S. R. HALL, U.S.D.A. Endocrinology, *34*, No. 1: 1. Jan., 1944.

The slope of the regression line in the systemic crop-weight method of lactogen assay with 6-week-old White Carneau pigeons was valid only when stimulation produced crop-sacs weighing more than 2200 mg. Below this level a flatter curve resulted. Muscle extracts added to purified lactogen solutions in certain proportions augmented the crop-sac response and in other proportions depressed it. Subcutaneously administered lactogen produced about 20 per cent greater response than did intramuscularly administered lactogen. Data on the response of 5 different lactogen preparations tested on 561 six-week-old White Carneau pigeons 53 different times during a period of one year showed wide variation in response to the same

amount of the same preparation. The time-to-time variation was less with crop-sac responses below 2300 mg. R.P.R.

157. Effect of Thiouracil and Thiourea on the Thyroid Gland of the Chick. J. P. MIXNER, E. P. REINEKE, AND C. W. TURNER, University of Missouri. Endocrinology, *34*, No. 3: 168. Mar., 1944.

Chick thyroid enlargement was found to occur following the feeding of either thiouracil or thiourea. Approximately 0.1 per cent thiouracil or thiourea in the diet for 14 days was the optimal dosage for the production of maximal enlargement of the thyroid in one to 2-day-old White Plymouth Rock chicks. Female chicks exhibited a greater thyroid response than did male chicks. Increasing dosages of thyroxine progressively decreased the degree of thyroid enlargement, female chicks requiring greater amounts. Nine breeds of chickens were given 0.1 per cent thiouracil in drinking water. The thyroids of the lighter breeds of chickens gave a greater response than did those of the heavier breeds. The application of the experimental results in the formulation of a biological assay for materials having thyroxine-like activity was discussed. R.P.R.

MISCELLANEOUS

158. Vermin Control. CAPTAIN PAUL W. REED, Sn.C. Natl. Butter and Cheese Jour., *36*, No. 3: 28. Mar., 1945.

Cockroaches thrive in a warm, moist environment where food is available. They hide in dark places during the day. These hiding places should be dusted with the poison, sodium fluoride, once a week. Of other methods of control, trapping is not successful, baits containing phosphorus or arsenic may be used where dusting is objectionable, pyrethrum sprays are effective but not available, and DDT, which is not available, is no more effective than sodium fluoride. Rodent control is achieved by good housekeeping, by rat-proofing of buildings, and by baiting, trapping, and fumigating. Successful poisons are barium carbonate and red squill. One part of the poison is effective when mixed with 16 parts of hamburger, oatmeal, cheese, or other bait and when enough bait is provided in small paper packages for all rats. Snap traps, securely baited and anchored in or near runways are good. For rodents and cockroaches, fumigation is effective but expensive, and dangerous unless handled by skilled operators. W.V.P.

159. Outlook for Aluminum in the Dairy Industry. ANONYMOUS. Natl. Butter and Cheese Jour., *36*, No. 3: 32. Mar., 1945.

Use of aluminum will probably increase because it is cheaper, more plentiful, and because men are now more skilled in working the metal.

Aluminum is widely used here and abroad, for milk cans, storage tanks, churns, heat exchangers and the like. Its use in foil for bottle caps and wrappers for dairy products is well known. Corrosion of aluminum can be prevented by the use of proper cleaning methods and by eliminating "galvanic action" caused by other metals or solutions of metals coming in contact with the aluminum. Pictures show approved methods of brazing, insulating junctions of metals, and some construction details on tanks. A table lists fabrication materials (castings, sheets, etc.), alloys and finishes for 19 different uses of this metal in the dairy industry. W.V.P.

160. Six Roads to Bigger Dairy Products Sales. ANONYMOUS. Natl. Butter and Cheese Jour., *36*, No. 3: 30. Mar., 1945.

The National Dairy Council enlists the support of doctors, dentists, dieticians, nurses, nutritionists and public health officials. These professions largely determine the national food habits. Staff members of the Council keep in constant touch with the professional organizations and their publications. To these professional groups they furnish educational material, educational advertising and suitable movies. Nationwide publicity is created for newspapers, magazines, radio editors and others. Finally, exhibits and displays are designed to tell the essential place of dairy products in human nutrition. By these activities the council proposes to keep consumers wanting dairy products. W.V.P.

161. Post-War Plant Controls and Devices for the Dairy Industry. R. E. OLSON, Taylor Instrument Co., Rochester, N. Y. Natl. Butter and Cheese Jour., *36*, No. 3: 26. Mar., 1945.

New devices will be used only if they are reliable and save money. Temperature control devices will be improved for batch and continuous flow pasteurizers. Flow meters, instruments to show tank contents and pressures can be expected. Can washing can be improved by better control of temperatures and strength of cleaning solutions; this control can be attained by the aid of recording devices. A centralized control of important plant operations from a single panel board is now possible. Trends are toward continuous processing operations which depend on precise control instruments. W.V.P.

162. Practical Suggestions on Cleaning and Sterilizing. C. M. MOORE, Diversey Corp., Chicago, Ill. Natl. Butter and Cheese Jour., *36*, No. 2: 17. Feb., 1945.

A planned sanitary program should teach the dairy plant worker the sanitary significance, purposes, methods, and mechanics of proper cleaning. Typewritten instructions should be provided to tell how to clean and dis-

infect each major piece of equipment, and how to care for floors, walls, windows, storage rooms, and the like. This discussion includes such practical suggestions as: rinsing of equipment after using to make cleaning easier; cleaning thoroughly before disinfection; and using 170° F. water carefully to prevent damage to glass and buckling of steel vats. W.V.P.

163. Apprentice Training in the Dairy Industry. WALTER F. SIMON, Indus. Commission of Wis., Madison, Wis. Nat. Butter and Cheese Jour., *35*, No. 10: 20. Oct., 1944.

Apprentice training in the dairy industry should be more widely used. There should be a contract between employer and apprentice that should specify term of training, kind of work the apprentice will do, the amount and kind of supplementary schooling, and wages to be paid the apprentice during training. Organized labor favors this system. Returning veterans may be particularly interested in apprentice training because they will receive an allowance from the government in addition to wages.

W.V.P.

164. Acetylated Casein Fiber. ALFRED E. BROWN, W. G. GORDON, EDITH C. GALL, AND R. W. JACKSON, Eastern Regional Research Laboratory, U. S. Dept. of Agr., Philadelphia, Pa. Indus. and Engin. Chem. Indus. Ed., *36*, No. 12, 1171. Dec. 1944.

Casein fiber as ordinarily hardened with formaldehyde is inferior to wool as a textile fiber because of its lower tensile strength, especially when wet and because of its poor resistance to boiling, particularly in mildly alkaline solutions. Acetylation carried out with acetic anhydride under various conditions produced fibers of acetyl contents of 1 to 9% which showed various properties. Acetyl contents of about 6% could be introduced with no loss of wet or dry tensile strength. These fibers were superior to the untreated fibers in regard to resistance to boiling solutions simulating dye-bath conditions and they more closely approximated wool in dye uptake.

B.H.W.

165. Dairy Cleaners and Cleaning Dairy Equipment. W. L. MALLMANN, Mich, Agr. Expt. Sta. Milk Plant Monthly, *33*, No. 11: 40–42. Nov., 1944.

With the possible exception of personnel, cleaning dairy equipment and the type of cleaner used are probably the most important factors in the production of quality milk. Although cleaning is largely a matter of time and effort on the part of the operator, the type and amount of cleaner are secondary, but aid materially in speed and ease of cleaning. Cleaned dairy equipment is free from soil and chemical substance, appearing bright and

shiny and shedding water in an even sheet. Disassembling of equipment after each use should be a part of good dairy cleaning. Brushing aids materially and speeds the cleaning action of even the best compounds. Washing water should be at a temperature of 115° to 122° F., in order to remove the soil more easily. A good cleaner frees the surfaces of fats, proteins and salts without the formation of curd. The cleaner should dissolve in wash water without formation of floc, leaving the mixture crystal clear. Fat removal is accomplished by saponification or emulsification. Wetting agents, lowering the surface tension of the cleaning solution, cause the soil to wet rapidly, thus allowing the cleaner to act quickly. Alkaline cleaners at pH 10.5 are good deflocculating and protein-dissolving agents. Their value is lost to a large extent when the pH values are above or below this point. The hardness of water affects the action of a cleaner. The harder the water, the more the detergent used up in the softening process. Hardness of water can be removed by rendering the calcium and magnesium ineffective by use of compounds such as pyrophosphates, tetraphosphates and hexaphosphates. Milk and water stone can be avoided by using cleaners containing these compounds. Acid rinse or acidified steam is used largely as a milk stone preventive. If equipment is properly washed and sanitized, bacterial activity should be completely stopped. G.M.T.

166. **What Lies Ahead for Milk Dealers and Supplymen?** JOHN W. LADD, President Dairy Industries Supply Assoc. Milk Dealer, *34*, No. 1: 56–58. Oct., 1944.

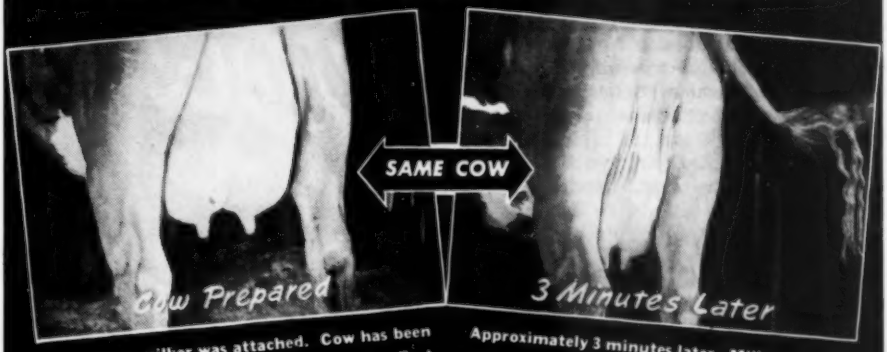
Despite the phenomenal total of 118,000,000,000 pounds of milk produced it has been processed without spoilage in the face of curtailed labor, old equipment, no new machinery and increased costs due to restrictive regulations. With the nutritional impetus given to milk and milk products under war-time utilization, post-war consumption will forge ahead with consequent expansion of the milk industry, the supply industry and dairy products processing generally. With the unparalleled backlog of supplies needed in every branch of the dairy industry caused by the demands of war-time emergencies, the first post-war equipment and machinery for the dairy industry will largely be of pre-war models to be followed as reconversion plans can be made by many new and interesting materials and processes which will ultimately insure finer dairy products. C.S.T.

167. **Controlling Insects—A Problem in the Dairy Industry.** LLOYD K. RIGGS, Kraft Cheese Co., Chicago, Ill. Milk Dealer, *33*, No. 11: 58–60. Aug., 1944.

Elimination of insect infestation in milk-processing plants is a tough, steady job requiring expert knowledge. Not one plant operator in ten

possesses such knowledge. No visible evidence of insect infestation in a milk plant is not a solution of the problem. The eradication of invisible infestation is a "must" project in a properly operated and sanitary milk or dairy plant. To solve the problem frequently involves major repairs in the building and equipment, engineering and architectural corrections of buildings or even the building of a new plant or factory. The control of insects in all food-processing plants is highly important and deserves careful consideration in the erection of buildings and installation of all equipment. Under existing war-time condition, continuous control methods by use of high potency sprays and fumigation are a "must" program if insect infestation is to be left under control and if possible eradicated. C.S.T.

The DE LAVAL MAGNETIC *SPEEDWAY* MILKER



Just before milker was attached. Cow has been prepared according to De Laval Speedway Fast Milking Method. Note distention of udder indicating full milk "let-down."

Approximately 3 minutes later. Milker removed and De Laval fast, uniform milking has obtained the milk quickly and completely, saving time and labor and protecting udder health.

FAST and *UNIFORM* MILKING Does It!

The graphic pictures above tell the story of fast, clean and complete milking results . . . obtained with the *fast* and *uniform* milking of the De Laval Magnetic Speedway Milker and the De Laval Speedway Method of Fast Milking.

Results . . . and years of experience *prove* that both *fast* and *uniform* milking are necessary to best milking results. Only the De Laval Magnetic Speedway Milker provides *both* these essential qualities.

And results . . . on thousands of farms are likewise proving the value of the De Laval Speedway Method of Fast Milking—proper preparation of the cow before milking and proper operation of the milker itself.

If fast, clean and complete milking is what *you* want . . . please talk with your local De Laval Dealer.

DE LAVAL STERLING MILKER

The De Laval Sterling Milker is particularly adapted for those to whom lower price is an important consideration. The Sterling Pulsator has only two moving parts, gives positive milking speed and action that pleases the cow. De Laval Sterling single or double units may also be used on any other make of single pipe line installation.

DE LAVAL SEPARATORS

De Laval Cream Separators skim cleaner, last longer, cost less per year of use and earn more. They produce highest quality cream and may easily be washed in a few minutes' time under ordinary farm conditions. Made in a wide variety of sizes and styles and at prices to meet every need and purse. Hand or motor drive.

THE DE LAVAL SEPARATOR COMPANY

NEW YORK 6 — 165 BROADWAY
CHICAGO 6 — 427 RANDOLPH ST
SAN FRANCISCO 19 — 61 BEALE ST

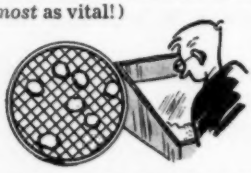
Your advertisement is being read in every State and in 25 Foreign Countries

HOW TO SEPARATE A CAT FROM A MOUSE

● Simply a matter of screening . . . a useful trick for a mouse to know. In fact, screening is a useful trick in any trade . . . even the salt business!

You see, in the salt business we've got to fit the salt to the job. Butter-makers don't want large, slow-dissolving crystals in Butter Salt. We remove the "big ones" so completely, you won't find even a trace on a 28-mesh Tylor screen. But they don't want fine dust, either, to cause pasting in the churn. Diamond Crystal Butter Salt contains only 3% of particles small enough to pass through a 65-mesh screen!

Yes, it's as vital to the butter-maker that we remove over-sized and under-sized salt crystals . . . as it is to the mouse to screen out that cat. (Well, *almost* as vital!)



Happily for the mouse, he got results. And so do we at Diamond Crystal. That's why you can always be sure of clean screening whenever you specify Diamond Crystal. Take your choice of grade or grain size—it's tops by actual tests!

Want Free Information on Salt? Write Us!

If *you* have a salt problem, let our Technical Director help! Just drop him a line in care of Diamond Crystal Salt, Dept. H-11, St. Clair, Michigan.

DIAMOND CRYSTAL
ALBERGER PROCESS **SALT**

to the very last drop

When a Seal-Kap goes over the pouring lip of a clear glass bottle, it is there to protect the contents right through to the end.

During handling and delivery, the pouring lip is completely shielded from danger of contaminating contacts.

In the home, Seal-Kaps come off the bottle with an easy twist of the wrist, avoiding fork-prying and splashing hazards.

And tough, durable Seal-Kaps go back on, as often as necessary, with a tight-fitting snap. As long as there is milk in the bottle, it is safe from spilling and danger of adjacent food taints.

With Seal-Kap protection, pure milk stays pure, right down to the last drop. Now, more than ever, they guard the nation's vital milk supplies.

AMERICAN SEAL-KAP CORPORATION
11-05 44th Drive, Long Island City, N. Y.
SEAL-KAP COVERS THE DANGER ZONE

Your advertisement is being read in every State and in 25 Foreign Countries

Our bulletin 30c is fully descriptive—Write for it.

"THE GAULIN TWO STAGE HOMOGENIZER AND HIGH PRESSURE PUMPS have met the Dairy Industries needs for a durable, heavy duty and economical machine, since 1909. Homogenizers and Pumps are still available with proper priority ratings, as well as repair and replacement parts. Both machines are strictly sanitary and are approved by all leading health boards. The Homogenizers are built in sizes from 75 gallons to 2000 gallons per hour, and the High Pressure Pumps are custom built to suit individual needs. The Gaulin is the ideal, all-purpose machine for fluid milk, ice cream, evaporated milk, condensed milk and spray-drying milk or eggs."

MANTON-GAULIN
TWO STAGE
HOMOGENIZER

THE MANTON-GAULIN MFG. CO., INC.
7 CHARLTON STREET EVERETT, MASS., U.S.A.

New Slant on CALF SCOURS

Extensive research at the Wisconsin Agricultural Experiment Station shows that most cases of calf scours are due to a dietary deficiency. The contributing factors are vitamin A and niacin. The same formula used in the Wisconsin studies for preventing and curing nutritional scours has been employed in ANADEX Kaf-Kaps.

Daily use of ANADEX for the first few weeks is helping dairymen save calves from scours and attendant disorders. Calves on skimmilk diets receive in ANADEX adequate vitamin A till they are eating freely of hay and grain. The niacin requirements are supplied till the calf begins to synthesize this nutrient. In addition adequate vitamin D is provided for rapid growth and strong skeletal development.

ANADEX Kaf-Kaps are 10 minim (⅔ cc) capsules. Easy to administer . . . no loss in feeding or in potency. It requires about 25 Kaps per calf, at a cost to the dairyman of 73¢ to $1.00 depending on size of package. Inquiries invited.

NUTRITION PRODUCTS CO.
AURORA, ILLINOIS

FLAV-O-LAC FLAKES

THE CULTURE

of definitely better flavor & aroma-producing qualities.

The standard with foremost operators, agricultural schools & colleges.

FLAV-O-LAC FLAKES (shown) produce a quart of the finest starter on a single propagation. Single bottles $2.00.

SPECIAL FLAV-O-LAC FLAKES "40" produce 40 quarts of starter on a single propagation. Single bottles $3.00

Free Culture Manual of Fermented Milk Products on request.

Pioneers in Spectro-chemical, Chemical and Fluoro-photometric Determinations of Vitamins A, B_1, B_2, Nicotinic Acid, Pantothenic Acid, B_6, C & E in Dairy and Food Products. (Vitamin D excluded) inquiries invited.

THE DAIRY-LABORATORIES
DIVISION DALARE ASSOCIATES
23rd & Locust Sts., Phila., Pa.
BRANCHES
New York Baltimore Washington
See our catalog in Dairy Industries Catalog.

Your advertisement is being read in every State and in 25 Foreign Countries

True Sanitation Begins BEFORE Milking!

Proper sanitation begins with the cow. It is an important contribution toward improving the quality of milk and milk products. Realizing this, more and more dairy men are turning to

OAKITE BACTERICIDE

for the effective . . . economical germicidal treatment of udder wash water and milking machine teat cup rinse water.

They are finding that Oakite Bactericide, because of its more active form of available chlorine, quickly destroys bacterial organisms . . . helps keep bacterial counts LOW. This performance-proved material is completely soluble, drains freely and leaves no white residues on equipment. Write for FREE 8-page booklet giving details!

OAKITE PRODUCTS, INC.,
Dairy Research Division
16G THAMES STREET, NEW YORK 6, N. Y.
Technical Service Representatives Located in All Principal Cities of the United States and Canada

OAKITE CLEANING

"HANSEN'S" Dairy Preparations

Cheese Rennet and Color
Annatto Butter Color
Certified Butter Color
Ice Cream Color
Lactic Ferment Culture
Bulgarian Culture

—★—

Odorless Dairy Fly Spray
Testing Solutions
Rennet Tests

CHR. HANSEN'S LABORATORY, INC.
MILWAUKEE, WISCONSIN

Promise . . . .

It's the time of promise. Of great events in the world. New realizations, sounder objectives. All that has been learned must be put to work. • Of promise, too, for the dairy industries . . . in restorations and a going forward. • Members of DISA are planning and preparing. They will always work with you closely. . . .

DAIRY INDUSTRIES SUPPLY ASSOCIATION

Albee Building Washington 5, D. C.

Your advertisement is being read in every State and in 25 Foreign Countries

NU-KLEEN

ORGANIC DETERGENT

For All Types of Equipment Cleaning by Hand

Simplify your cleaning problem by using this new effective organic detergent especially balanced for all hand cleaning operations. NU–KLEEN is one of today's most popular "acid" cleaners. Its organic acid content is skillfully adjusted to the wetting agent concentration providing the ideal amount of penetration and coverage. NU–KLEEN removes and prevents milkstone. It is free rinsing and leaves no film or other deposits. You can brush or spray NU–KLEEN with equally good results and do away with laborious old time cleaning methods. NU–KLEEN is non-corrosive and harmless to hands and clothing. Keep a supply on hand always for plant use and for your patrons' milking utensils and milking machines.

KLENZADE PRODUCTS, INC.
Beloit, Wisconsin

Marschall
RENNET and COLOR

For best results in the cheese vat, specify Marschall's. High in strength, pure and uniform.

MARSCHALL DAIRY LABORATORY, INC.
MADISON 3, WISCONSIN

D-LUXE

LEAVES NO "FRECKLES" ON DAIRY EQUIPMENT

Freckles may be an asset to a smiling youngster, but on dairy equipment they can be a decided handicap. We mean those white deposits and water spots that so often show up on sanitary piping and other bright surfaces after cleaning . . . especially with hard water.

That's one reason why more and more dairy plants are turning to D–LUXE these days. D–LUXE provides a new and different type of water-softening action that assures trouble-free cleaning . . . a unique free-rinsing action that produces a spot-free, "like new" appearance to all surfaces.

Furthermore, D–LUXE goes after dirt on pasteurizing vats, sanitary pipe lines and fittings, tanks and other equipment with a powerful emulsifying action that literally shatters grease . . . breaks it up into tiny globules which float in the cleaning solution (makes an emulsion just like butterfat in milk)!

Efficiently cleaned with D–LUXE, dairy equipment can be made to last longer . . . work better . . . can meet the urgent demands for greater output.

THE DIVERSEY CORPORATION,
53 West Jackson Blvd., Chicago 4, Ill.

Your advertisement is being read in every State and in 25 Foreign Countries

"El progreso del mundo"—un discusión entre el Sr. Miguel y su caballo, Plato.

"You Know Something, Plato? The World, She Moves Ahead"

DON MIGUEL—"For example, take that bit you have in your mouth. It is of soft, polished steel. It will not break, yet it will not cut you. Your four-footed forefathers, mi amigo, had bits made of madero. Wood, you understand?"

PLATO—"Would I understand, he asks me. Sí, señor. A bit of progress, no?"

DON MIGUEL—"You make the joke, Plato. But here is un otro bit of progress. When the insect he comes, you swish the tail, you scratch the rump against the tree. Is it not so? Now, this Science he brings you the wonderful insect killer, D.D.T. After the war, plenty of D.D.T. to kill the insects. You will like that very much, amigito."

PLATO—"Will it give me the D.D.T.'s?"

DON MIGUEL—"Un momentito, not so fast. I will tell to you something else. I learn it at Hacienda Miguel. How Nature and Science, they work together, to develop the finest vanilla flavoring the world she has ever seen.

"Those Mayas and Aztecs . . . never did they see such vines in the jungle as we have growing in our vanillery at Mayaguëz, clinging to supporting pipes in the soft, tropical shade. We tend them like the mother at the baby's crib. And with chemicals that are lacking in the soil.

"Their lovely vanilla pods are sent to the Michael's laboratory in Philadelphia. Science he continues una otra vez (once more) where Nature she stops. You follow me, Plato? The vanilla is enhanced with vanillin derived from tropical spices, to bring Michael's Mixevan under the spell of *controlled flavor*."

PLATO—"They have a sense of proportion."

DON MIGUEL—"You can say that again. And this *controlled flavor* in Mixevan, old caballo, enables the ice cream manufacturer to have *controlled flavor* when he makes the delicious vanilla ice cream.

"Soon, Plato, we will have the story of *controlled flavor* in booklet form so that it can be given to the millions of people who always want to know more about America's Favorite —ICE CREAM."

PLATO—"Hay, hay. And how about feeding me some?"

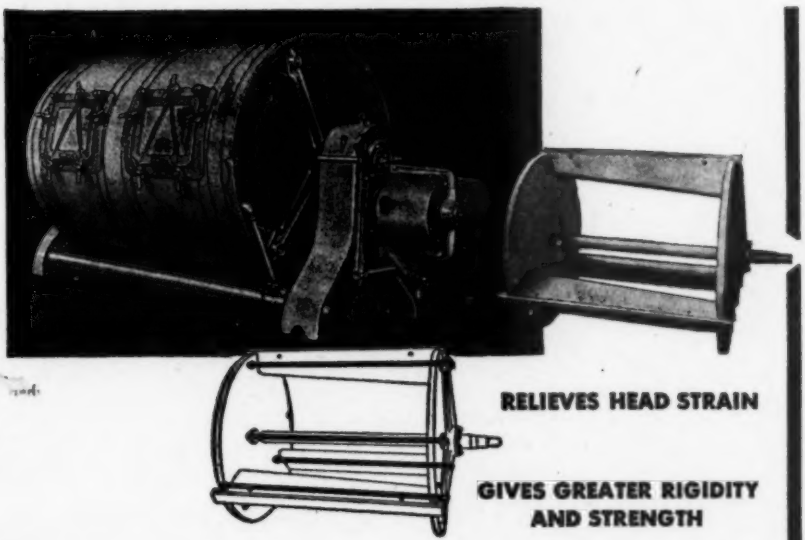
Mixevan—"America's Flavorite" and other powdered vanilla products

DAVID MICHAEL & CO.

half a century in the flavoring field

Front and Master Streets, Philadelphia 22, Pa.

CP's *Exclusive* SEALED-IN TIE-ROD CONSTRUCTION



RELIEVES HEAD STRAIN

GIVES GREATER RIGIDITY AND STRENGTH

WHEN you choose a CP Sanitary No-Roll Churn with its exclusive sealed-in tie-rod features, you get the strongest and most sanitary construction possible. Note from illustration above how the tie-rods—entirely sealed away from churn interior — extend through the shelves and are fastened outside of the gudgeon arms. Center working bar also has the sealed-in tie-rod feature.

Unexcelled performance — the tapered shelves and center working bar work the product the full length of drum and provide uniform composition.

Buttermakers like the fact that with the CP No-Roll Churn they can work the butter hard or soft without its getting sticky . . . obtaining a dry, firm waxy textured butter which GIVES MAXIMUM YIELD WHEN PRINTED. Ask for Bulletin K-689.

THE CREAMERY PACKAGE MFG. COMPANY, 1243 W. Washington Blvd., Chicago 7, Ill.
Branches: Atlanta—Boston—Buffalo—Chicago—Dallas—Denver—Kansas City
Los Angeles — Minneapolis — New York — Omaha — Philadelphia — Portland, Oregon — Salt Lake City — San Francisco — Seattle — Toledo — Waterloo, Iowa
CREAMERY PACKAGE MFG. CO. OF CANADA, Ltd., 267 King St. West, Toronto, Ont., Canada

4TH WAR LOAN

Your advertisement is being read in every State and in 25 Foreign Countries

DIFCO

Detection of
MOLDS *and* YEASTS
in Dairy Products

Bacto-Potato Dextrose Agar is recommended for determination of the mold and yeast count of butter. This product is also used extensively for isolation and cultivation of molds and yeasts in other dairy products.

Bacto-Potato Dextrose Agar is prepared according to the formula specified in "Standard Methods for the Examination of Dairy Products" of the American Public Health Association. Medium prepared from the dehydrated product conforms in every way to the standard medium. After sterilization the medium will have a reaction of pH 5.6 which may readily be adjusted to pH 3.5 by addition to sterile tartaric acid.

Bacto-Malt Agar is also an excellent medium for detection of molds and yeasts in butter. This medium is widely used for determining the mold and yeast count of butter and other dairy products, and is particularly useful in revealing sources of contamination by these organisms.

Bacto-Malt Agar is readily prepared and has a reaction of pH 5.5 after sterilization in the autoclave. This reaction may readily be adjusted to pH 3.5 by addition of lactic acid.

Specify "DIFCO"

THE TRADE NAME OF THE PIONEERS
In the Research and Development of Bacto-Peptone and Dehydrated Culture Media

DIFCO LABORATORIES
INCORPORATED
DETROIT 1, MICHIGAN

Your advertisement is being read in every State and in 25 Foreign Countries